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(71) Applicant (for all designated States except US): QUARK BIOTECH, INC. [US/US]; 1059 Serpentine Lane, Pleasanton, CA 94566 (US).			
(71) Applicant (for MW only): KOHN, Kenneth, I. [US/US]; 6761 Alderly Way, West Bloomfield, MI 48322 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): EINAT, Paz [IL/IL]; Apartment 27, 1 Nave Nir, 74402 Nes Ziona (IL). SKALITER, Rami [IL/IL]; Apartment 10, 117 Habanim Street, 74037 Nes Ziona (IL).			
(74) Agents: KOHN, Kenneth, I. et al.; Kohn & Associates, Suite 410, 30500 Northwestern Highway, Farmington Hills, MI 48334 (US).		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: HYPOXIA-REGULATED GENES			
(57) Abstract <p>According to the present invention, purified, isolated and cloned nucleic acid sequences encoding hypoxia-regulating genes and the proteins thereof and antibodies directed against the proteins which have sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 are provided. The present invention further provides transgenic animals and cell lines as well as knock-out organisms of these sequences. The present invention further provides methods of regulating angiogenesis or apoptosis or regulating response to hypoxic conditions in a patient in need of such treatment. The present invention also provides a method of diagnosing the presence of ischemia in a patient including the steps of analyzing a bodily fluid or tissue sample from the patient for the presence or gene product of at least one expressed gene (up-regulated) as set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No: 4; SEQ ID No:5; and SEQ ID No:6 and where ischemia is determined if the up-regulated gene or gene product is ascertained.</p>			

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HYPOXIA-REGULATED GENES

BACKGROUND OF THE INVENTION

5

1. FIELD OF THE INVENTION

Identification of genes that are differentially expressed in hypoxia and use of the genes and gene products for diagnosis and therapeutic intervention.

10

2. DESCRIPTION OF RELATED ART

The level of tissue oxygenation plays an important role in normal development as well as in pathologic processes such as ischemia. Tissue oxygenation plays a significant regulatory role in both apoptosis and in angiogenesis (Bouck et al, 1996; Bunn et al, 1996; Dor et al, 1997; Carmeliet et al, 1998). Apoptosis (see Duke et al, 1996 for review) and growth arrest occur when cell growth and viability are reduced due to oxygen deprivation (hypoxia). Angiogenesis (i.e. blood vessel growth, vascularization), is stimulated when hypooxygenated cells secrete factors which stimulate proliferation and migration of endothelial cells in an attempt to restore oxygen homeostasis (for review see Hanahan et al, 1996).

25

Ischemic disease pathologies involve a decrease in the blood supply to a bodily organ, tissue or body part generally caused by constriction or obstruction of the blood vessels as for example retinopathy, acute renal failure, myocardial infarction and stroke. Therefore apoptosis and angiogenesis as induced by the ischemic condition are also involved in these disease states. Neoangiogenesis is seen in some forms of retinopathy and in tumor growth. It is recognized that angiogenesis is necessary for tumor growth and that retardation of angiogenesis would be a useful tool in controlling malignancy and retinopathies. Further, it would be useful to induce tumorigenic cells to undergo apoptosis (i.e. programmed cell death).

However, these processes are complex cascades of events controlled by many different genes reacting to the various stresses such as hypoxia. Expression of different genes reacting to the hypoxic stress can trigger not only apoptosis or angiogenesis but both. In cancer it has been observed that apoptosis and angiogenesis related genes are therapeutic targets. However, hypoxia itself plays a critical role in the selection of mutations that contribute to more severe tumorigenic phenotypes (Graeber et al., 1996). Therefore identifying candidate genes and gene products that can be utilized therapeutically not only in cancer and ischemia

and that may either induce apoptosis or angiogenesis or to retard the processes is needed. It would be useful to identify genes that have direct causal relationships between a disease and its related pathologies and an up-
5 or down-regulator (responder) gene.

SUMMARY OF THE INVENTION

According to the present invention, purified,
10 isolated and cloned nucleic acid sequences encoding hypoxia-responding genes which have sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4 and SEQ ID No:5 or a complementary or allelic variation sequence and human
15 homologs as needed thereto. The present invention further provides proteins as encoded by the nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 with SEQ ID Nos:7-11 being exemplars of the proteins. The
20 present invention further provides antibodies directed against the proteins as encoded by the nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 including SEQ ID Nos:7-11.

25 The present invention further provides transgenic animals and cell lines carrying at least one expressible

nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6. The present invention further provides knock-out eucaryotic organisms in which at least one nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 is knocked-out.

The present invention provides a method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the nucleic acid sequences as set forth in SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6. Alternatively, the present invention provides a method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one antisense oligonucleotide against the nucleic acid sequences as set forth in SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 or dominant negative peptide directed against the sequences or their proteins.

The present invention further provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a protein enclosed by SEQ ID Nos:2-6 or the protein sequences as set forth in

SEQ ID Nos:7-8,10-11 as active ingredients in a pharmaceutically acceptable carrier.

The present invention provides a method of providing an apoptotic regulating gene by administering directly to
5 a patient in need of such therapy utilizing gene therapy an expressible vector comprising expression control sequences operably linked to one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5 and SEQ ID No:6 (human homolog).

10 The present invention also provides a method of providing an angiogenesis regulating gene utilizing gene therapy by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the sequences
15 set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5 and SEQ ID No:6.

The present invention provides a method of regulating response to hypoxic conditions in a patient in need of such treatment by administering to a patient a
20 therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6. The present invention further provides a method of
25 providing a hypoxia regulating gene utilizing gene therapy by administering directly to a patient in need of

5 The present invention also provides a method of
diagnosing the presence of ischemia in a patient
including the steps of analyzing a bodily fluid or tissue
sample from the patient for the presence or gene product
of at least one expressed gene (up-regulated) as set
10 forth in the group comprising SEQ ID No:2; SEQ ID No:3;
SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 and where
ischemia is determined if the up-regulated gene or gene
product is ascertained.

15 DESCRIPTION OF THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

FIGURE 1 is a computer scan showing in-vitro translation of Full length cDNA clones of RTP801 (SEQ ID No:1). cDNA clones were translated in-vitro in using a coupled transcription translation kit (Promega). Translation products were separated on acrylamide gel and

exposed to X-ray film. Two clones, marked with arrows, gave the expected protein size of approximately 30KD. This confirms the sequence analysis of the putative reading frame.

5 FIGURE 2 is a computer scan showing RTP801 (SEQ ID No:1) Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, blotted onto
10 Nytran membranes and hybridized with rtp241 probe. One band of 1.8Kb is observed showing a marked induction after hypoxia

 FIGURE 3 is a computer scan showing RTP779 (SEQ ID No:2) Northern blot analysis. RNA was extracted from Rat
15 C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, blotted onto Nytran membranes and hybridized with rtp779 probe. One
20 band of 1.8Kb is observed showing extreme differential expression.

 FIGURE 4 is a computer scan showing RTP241 (SEQ ID No:3) Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample
25 were separated on denaturing agarose gels, blotted onto Nytran membranes and hybridized with rtp241 probe. Two

bands of 1.8Kb and 4Kb are observed, both show good differential expression.

FIGURE 5 is a computer scan showing RTP359 (SEQ ID No:5) Northern blot analysis. RNA was extracted from Rat C6 glioma cells which were exposed to hypoxia for 0, 4, or 16 hours. PolyA+ selected mRNA (2ug) from each sample were separated on denaturing agarose gels, blotted onto Nytran membranes and hybridized with rtp359 probe. One band of 4.5Kb is observed showing good differential expression.

DETAILED DESCRIPTION OF THE INVENTION

The present invention identifies candidate genes and gene products that can be utilized therapeutically and diagnostically not only in hypoxia and ischemia and that may regulate apoptosis or angiogenesis. By regulate or modulate or control is meant that the process is either induced or inhibited to the degree necessary to effect a change in the process and the associated disease state in the patient. Whether induction or inhibition is being contemplated will be apparent from the process and disease being treated and will be known to those skilled in the medical arts. The present invention identifies genes for gene therapy, diagnostic and therapeutics that have direct causal relationships between a disease and

its related pathologies and up- or down-regulator (responder) genes. That is the present invention is initiated by a physiological relationship between cause and effect.

5 The present invention provides purified, isolated and cloned nucleic acid polynucleotides (sequences) encoding genes which respond at least to hypoxic conditions by up-regulation of expression and which have sequences as set forth in the group comprising SEQ ID
10 No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4 and SEQ ID No:5 and their analogues and polymorphisms or a complementary or allelic variation sequence thereto. The present invention further provides SEQ ID No:6 which is a known gene (neuroleukin) which also responds to the
15 stress of hypoxia by being up-regulated. SEQ ID No:6 is the human sequence for neuroleukin and has over 90% homology with the rat sequence. The human homolog is used where appropriate. Because of the high homology between the rat and human sequences the rat sequence can
20 also be used for probes and the like as necessary.

 The present invention further provides proteins and their analogues as encoded by the nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 with SEQ ID Nos:7 and 8 as well
25 as SEQ ID Nos:9-11 being exemplars of the proteins. The present invention further provides a method of regulating

angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a protein enclosed by SEQ ID Nos:2-6 or the protein sequences as set forth in SEQ ID Nos:7-8,10-11 as active ingredients in a pharmaceutically acceptable carrier.

The proteins may be produced recombinantly (see generally Marshak et al, 1996 "Strategies for Protein Purification and Characterization. A laboratory course manual." CSHL Press) and analogues may be due to post-translational processing. The term Analogue as used herein is defined as a nucleic acid sequence or protein which has some differences in their amino acid/nucleotide sequences as compared to the native sequence of SEQ ID Nos:1-8. Ordinarily, the analogue will be generally at least 70% homologous over any portion that is functionally relevant. In more preferred embodiments the homology will be at least 80% and can approach 95% homology to the protein/nucleotide sequence. The amino acid or nucleotide sequence of an analog may differ from that of the primary sequence when at least one residue is deleted, inserted or substituted, but the protein or nucleic acid molecule remains functional. Differences in glycosylation can provide protein analogues.

Functionally relevant refers to the biological property of the molecule and in this context means an *in vivo* effector or antigenic function or activity that is directly or indirectly performed by a naturally occurring protein or nucleic acid molecule. Effector functions include but are not limited to include receptor binding, any enzymatic activity or enzyme modulatory activity, any carrier binding activity, any hormonal activity, any activity in promoting or inhibiting adhesion of cells to extracellular matrix or cell surface molecules, or any structural role as well as having the nucleic acid sequence encode functional protein and be expressible. The antigenic functions essentially mean the possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against a naturally occurring protein. Biologically active analogues share an effector function of the native which may, but need not, in addition possess an antigenic function.

The present invention further provides antibodies directed against the proteins as encoded by the nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 which can be used in immunoassays and the like.

The antibodies may be either monoclonal, polyclonal or recombinant. Conveniently, the antibodies may be prepared against the immunogen or portion thereof for

example a synthetic peptide based on the sequence, or prepared recombinantly by cloning techniques or the natural gene product and/or portions thereof may be isolated and used as the immunogen. Immunogens can be used to produce antibodies by standard antibody production technology well known to those skilled in the art as described generally in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992. Antibody fragments may also be prepared from the antibodies and include Fab, F(ab')₂, and Fv by methods known to those skilled in the art.

For producing polyclonal antibodies a host, such as a rabbit or goat, is immunized with the immunogen or immunogen fragment, generally with an adjuvant and, if necessary, coupled to a carrier; antibodies to the immunogen are collected from the sera. Further, the polyclonal antibody can be absorbed such that it is monospecific. That is, the sera can be absorbed against related immunogens so that no cross-reactive antibodies remain in the sera rendering it monospecific.

For producing monoclonal antibodies the technique involves hyperimmunization of an appropriate donor with the immunogen, generally a mouse, and isolation of splenic antibody producing cells. These cells are fused

to a cell having immortality, such as a myeloma cell, to provide a fused cell hybrid which has immortality and secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies
5 harvested from the culture media for use.

For producing recombinant antibody (see generally Huston et al, 1991; Johnson and Bird, 1991; Mernaugh and Mernaugh, 1995), messenger RNAs from antibody producing B-lymphocytes of animals, or hybridoma are reverse-
10 transcribed to obtain complimentary DNAs (cDNAs). Antibody cDNA, which can be full or partial length, is amplified and cloned into a phage or a plasmid. The cDNA can be a partial length of heavy and light chain cDNA, separated or connected by a linker. The antibody, or
15 antibody fragment, is expressed using a suitable expression system to obtain recombinant antibody. Antibody cDNA can also be obtained by screening pertinent expression libraries.

The antibody can be bound to a solid support
20 substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific
25 Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the

art. (see for a general discussion Harlow & Lane
Antibodies: A Laboratory Manual, Cold Spring Harbor
Laboratory Publications, New York, 1988 and Borrebaeck,
Antibody Engineering - A Practical Guide, W.H. Freeman
5 and Co., 1992) The detectable moieties contemplated with
the present invention can include, but are not limited
to, fluorescent, metallic, enzymatic and radioactive
markers such as biotin, gold, ferritin, alkaline
phosphatase, β -galactosidase, peroxidase, urease,
10 fluorescein, rhodamine, tritium, ^{14}C and iodination.

The present invention further provides transgenic
animals and cell lines carrying at least one expressible
nucleic acid sequence as set forth in SEQ ID No:1, SEQ ID
No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID
15 No:6. By expressible is meant the inclusion with the
sequence of all regulatory elements necessary for the
expression of the gene or by the placing of the gene in
the target genome so that it is expressed. The present
invention further provides knock-out eucaryotic organisms
20 in which at least one nucleic acid sequences as set forth
in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4,
SEQ ID No:5 and SEQ ID No:6 is knocked-out.

These transgenics and knock-outs are constructed
using standard methods known in the art and as set forth
25 in United States Patents 5,487,992, 5,464,764, 5,387,742,
5,360,735, 5,347,075, 5,298,422, 5,288,846, 5,221,778,

5,175,385, 5,175,384, 5,175,383, 4,736,866 as well as
Burke and Olson (1991), Capecchi (1989), Davies et al.
(1992), Dickinson et al. (1993), Duff and Lincoln (1995),
Huxley et al. (1991), Jakobovits et al. (1993), Lamb et
5 al. (1993), Pearson and Choi (1993), Rothstein (1991),
Schedl et al. (1993), Strauss et al. (1993). Further,
patent applications WO 94/23049, WO 93/14200, WO
94/06908, WO 94/28123 also provide information.

More specifically, any techniques known in the art
10 may be used to introduce the transgene expressibly into
animals to produce the parental lines of animals. Such
techniques include, but are not limited to, pronuclear
microinjection (U.S. patent 4,873,191); retrovirus
mediated gene transfer into germ lines (Van der Putten et
15 al., 1985); gene targeting in embryonic stem cells
(Thompson et al., 1989; Mansour, 1990 and U.S. patent
5,614,396); electroporation of embryos (Lo, 1983); and
sperm-mediated gene transfer (Lavitrano et al., 1989).
For a review of such techniques see Gordon (1989).

20 Further, one parent strain instead of carrying a
direct human transgene may have the homologous endogenous
gene modified by gene targeting such that it approximates
the transgene. That is, the endogenous gene has been
"humanized" and/or mutated (Reaume et al, 1996). It
25 should be noted that if the animal and human sequence are
essentially homologous a "humanized" gene is not

required. The transgenic parent can also carry an overexpressed sequence, either the nonmutant or a mutant sequence and humanized or not as required. The term transgene is therefore used to refer to all these possibilities.

Additionally, cells can be isolated from the offspring which carry a transgene from each transgenic parent and that are used to establish primary cell cultures or cell lines as is known in the art.

Where appropriate, a parent strain will be homozygous for the transgene. Additionally, where appropriate, the endogenous nontransgene in the genome that is homologous to the transgene will be nonexpressive. By nonexpressive is meant that the endogenous gene will not be expressed and that this nonexpression is heritable in the offspring. For example, the endogenous homologous gene could be "knocked-out" by methods known in the art. Alternatively, the parental strain that receives one of the transgenes could carry a mutation at the endogenous homologous gene rendering it nonexpressed.

The present invention provides a method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of at least one protein as encoded by the nucleic acid sequences as set forth in

SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6. The antagonist is dosed and delivered in a pharmaceutically acceptable carrier as described herein below. The term antagonist or

5 antagonistizing is used in its broadest sense. Antagonism can include any mechanism or treatment which results in inhibition, inactivation, blocking or reduction in gene activity or gene product. It should be noted that the inhibition of a gene or gene product may provide for an

10 increase in a corresponding function that the gene or gene product was regulating. The antagonizing step can include blocking cellular receptors for the gene products of SEQ ID Nos:1-6 and can include antisense treatment as discussed herein below.

15 The present invention further provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a regulating agent the protein selected from the group consisting of SEQ ID

20 Nos:7-11 in a pharmaceutically acceptable carrier. The regulating agent is dosed and delivered in a pharmaceutically acceptable carrier as described herein below. For example, a patient may be in need of inducing apoptosis in tumorigenic cells or angiogenesis in trauma

25 situations where for example a limb must be reattached or in a transplant where revascularization is needed.

The present invention provides a method of regulating angiogenesis or apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one
5 antisense oligonucleotide or dominant negative peptide (either as cDNA or peptide; Herskowitz, 1987) directed against the nucleic acid sequences as set forth in SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6. The present invention also provides a
10 method of regulating response to hypoxic conditions in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID
15 No:1; SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6. The antisense oligonucleotide as the active ingredient in a pharmaceutical composition is dosed and delivered in a pharmaceutically acceptable carrier as discussed herein below.

20 Many reviews have covered the main aspects of antisense (AS) technology and its enormous therapeutic potential (Wright and Anazodo, 1995). There are reviews on the chemical (Crooke, 1995; Uhlmann et al, 1990), cellular (Wagner, 1994) and therapeutic (Hanania, et al,
25 1995; Scanlon, et al, 1995; Gewirtz, 1993) aspects of this rapidly developing technology. Within a relatively

short time, ample information has accumulated about the *in vitro* use of AS nucleotide sequences in cultured primary cells and cell lines as well as for *in vivo* administration of such nucleotide sequences for
5 suppressing specific processes and changing body functions in a transient manner. Further, enough experience is now available *in vitro* and *in vivo* in animal models and human clinical trials to predict human efficacy.

10 Antisense intervention in the expression of specific genes can be achieved by the use of synthetic AS oligonucleotide sequences (for recent reports see Lefebvre-d'Hellencourt *et al*, 1995; Agrawal, 1996; Lev-Lehman *et al*, 1997). AS oligonucleotide sequences may be
15 short sequences of DNA, typically 15-30 mer but may be as small as 7 mer (Wagner *et al*, 1996), designed to complement a target mRNA of interest and form an RNA:AS duplex. This duplex formation can prevent processing, splicing, transport or translation of the relevant mRNA.
20 Moreover, certain AS nucleotide sequences can elicit cellular RNase H activity when hybridized with their target mRNA, resulting in mRNA degradation (Calabretta *et al*, 1996). In that case, RNase H will cleave the RNA component of the duplex and can potentially release the
25 AS to further hybridize with additional molecules of the target RNA. An additional mode of action results from

the interaction of AS with genomic DNA to form a triple helix which may be transcriptionally inactive.

The sequence target segment for the antisense oligonucleotide is selected such that the sequence
5 exhibits suitable energy related characteristics important for oligonucleotide duplex formation with their complementary templates, and shows a low potential for self-dimerization or self-complementation [Anazodo et al., 1996]. For example, the computer program OLIGO
10 (Primer Analysis Software, Version 3.4), can be used to determine antisense sequence melting temperature, free energy properties, and to estimate potential self-dimer formation and self-complimentary properties. The program allows the determination of a qualitative estimation of
15 these two parameters (potential self-dimer formation and self-complimentary) and provides an indication of "no potential" or "some potential" or "essentially complete potential". Using this program target segments are generally selected that have estimates of no potential in
20 these parameters. However, segments can be used that have "some potential" in one of the categories. A balance of the parameters is used in the selection as is known in the art. Further, the oligonucleotides are also selected as needed so that analogue substitution do not
25 substantially affect function.

Phosphorothioate antisense oligonucleotides do not normally show significant toxicity at concentrations that are effective and exhibit sufficient pharmacodynamic half-lives in animals (Agarwal et al., 1996) and are

5 nuclease resistant. Antisense induced loss-of-function phenotypes related with cellular development were shown for the glial fibrillary acidic protein (GFAP), for the establishment of tectal plate formation in chick (Galileo et al., 1991) and for the N-myc protein, responsible for

10 the maintenance of cellular heterogeneity in neuroectodermal cultures (epithelial vs. neuroblastic cells, which differ in their colony forming abilities, tumorigenicity and adherence) (Rosolen et al., 1990; Whitesell et al., 1991). Antisense oligonucleotide

15 inhibition of basic fibroblast growth factor (bFGF), having mitogenic and angiogenic properties, suppressed 80% of growth in glioma cells (Morrison, 1991) in a saturable and specific manner. Being hydrophobic, antisense oligonucleotides interact well with

20 phospholipid membranes (Akhter et al., 1991). Following their interaction with the cellular plasma membrane, they are actively (or passively) transported into living cells (Loke et al., 1989), in a saturable mechanism predicted to involve specific receptors (Yakubov et al., 1989).

25 Instead of an antisense sequence as discussed herein above, ribozymes may be utilized. This is particularly

necessary in cases where antisense therapy is limited by stoichiometric considerations (Sarver et al., 1990, Gene Regulation and Aids, pp. 305-325). Ribozymes can then be used that will target the same sequence. Ribozymes are
5 RNA molecules that possess RNA catalytic ability (see Cech for review) that cleave a specific site in a target RNA. The number of RNA molecules that are cleaved by a ribozyme is greater than the number predicted by stoichiometry. (Hampel and Tritz, 1989; Uhlenbeck,
10 1987).

Ribozymes catalyze the phosphodiester bond cleavage of RNA. Several ribozyme structural families have been identified including Group I introns, RNase P, the hepatitis delta virus ribozyme, hammerhead ribozymes and
15 the hairpin ribozyme originally derived from the negative strand of the tobacco ringspot virus satellite RNA (STRSV) (Sullivan, 1994; U.S. Patent No. 5,225,347, columns 4-5). The latter two families are derived from viroids and virusoids, in which the ribozyme is believed
20 to separate monomers from oligomers created during rolling circle replication (Symons, 1989 and 1992). Hammerhead and hairpin ribozyme motifs are most commonly adapted for trans-cleavage of mRNAs for gene therapy (Sullivan, 1994). The ribozyme type utilized in the
25 present invention is selected as is known in the art. Hairpin ribozymes are now in clinical trial and are the

preferred type. In general the ribozyme is from 30-100 nucleotides in length.

Modifications or analogues of nucleotides can be introduced to improve the therapeutic properties of the nucleotides. Improved properties include increased
5 nuclease resistance and/or increased ability to permeate cell membranes.

Nuclease resistance, where needed, is provided by any method known in the art that does not interfere with
10 biological activity of the antisense oligodeoxy-nucleotides, cDNA and/or ribozymes as needed for the method of use and delivery (Iyer et al., 1990; Eckstein, 1985; Spitzer and Eckstein, 1988; Woolf et al., 1990; Shaw et al., 1991). Modifications that can be made to
15 oligonucleotides in order to enhance nuclease resistance include modifying the phosphorous or oxygen heteroatom in the phosphate backbone. These include preparing methyl phosphonates, phosphorothioates, phosphorodithioates and morpholino oligomers. In one embodiment it is provided
20 by having phosphorothioate bonds linking between the four to six 3'-terminus nucleotide bases. Alternatively, phosphorothioate bonds link all the nucleotide bases. Other modifications known in the art may be used where the biological activity is retained, but the stability to
25 nucleases is substantially increased.

The present invention also includes all analogues of, or modifications to, an oligonucleotide of the invention that does not substantially affect the function of the oligonucleotide. The nucleotides can be selected
5 from naturally occurring or synthetic modified bases. Naturally occurring bases include adenine, guanine, cytosine, thymine and uracil. Modified bases of the oligonucleotides include xanthine, hypoxanthine, 2-aminoadenine, 6-methyl, 2-propyl and other alkyl
10 adenines, 5-halo uracil, 5-halo cytosine, 6-aza cytosine and 6-aza thymine, psuedo uracil, 4-thiuracil, 8-halo adenine, 8-aminoadenine, 8-thiol adenine, 8-thiolalkyl adenines, 8-hydroxyl adenine and other 8-substituted adenines, 8-halo guanines, 8-amino guanine, 8-thiol
15 guanine, 8-thioalkyl guanines, 8-hydroxyl guanine and other substituted guanines, other aza and deaza adenines, other aza and deaza guanines, 5-trifluoromethyl uracil and 5-trifluoro cytosine.

In addition, analogues of nucleotides can be
20 prepared wherein the structure of the nucleotide is fundamentally altered and that are better suited as therapeutic or experimental reagents. An example of a nucleotide analogue is a peptide nucleic acid (PNA) wherein the deoxyribose (or ribose) phosphate backbone in
25 DNA (or RNA) is replaced with a polyamide backbone which is similar to that found in peptides. PNA analogues have

been shown to be resistant to degradation by enzymes and to have extended lives *in vivo* and *in vitro*. Further, PNAs have been shown to bind stronger to a complementary DNA sequence than a DNA molecule. This observation is attributed to the lack of charge repulsion between the PNA strand and the DNA strand. Other modifications that can be made to oligonucleotides include polymer backbones, cyclic backbones, or acyclic backbones.

The active ingredients of the pharmaceutical composition can include oligonucleotides that are nuclease resistant needed for the practice of the invention or a fragment thereof shown to have the same effect targeted against the appropriate sequence(s) and/or ribozymes. Combinations of active ingredients as disclosed in the present invention can be used including combinations of antisense sequences.

The antisense oligonucleotides (and/or ribozymes) and cDNA of the present invention can be synthesized by any method known in the art for ribonucleic or deoxyribonucleic nucleotides. For example, an Applied Biosystems 380B DNA synthesizer can be used. When fragments are used, two or more such sequences can be synthesized and linked together for use in the present invention.

The nucleotide sequences of the present invention

can be delivered either directly or with viral or non-viral vectors. When delivered directly the sequences are generally rendered nuclease resistant. Alternatively the sequences can be incorporated into expression cassettes
5 or constructs such that the sequence is expressed in the cell as discussed herein below. Generally the construct contains the proper regulatory sequence or promotor to allow the sequence to be expressed in the targeted cell.

Negative dominant peptide refers to a partial cDNA
10 sequence that encodes for a part of a protein, i.e. a peptide (see Herskowitz, 1987). This peptide can have a different function from the protein it was derived from. It can interact with the full protein and inhibit its activity or it can interact with other proteins and
15 inhibit their activity in response to the full protein. Negative dominant means that the peptide is able to overcome the natural proteins and fully inhibit their activity to give the cell a different characteristics like resistance or sensitization to killing. For
20 therapeutic intervention either the peptide itself is delivered as the active ingredient of a pharmaceutical composition or the cDNA can be delivered to the cell utilizing the same methods as for antisense delivery.

The present invention provides a method of providing
25 an apoptotic regulating gene, angiogenesis regulating gene or a hypoxia regulating gene by administering

directly to a patient in need of such therapy utilizing gene therapy an expressible vector comprising expression control sequences operably linked to one of the sequences set forth in the group comprising SEQ ID No:1; SEQ ID
5 No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

By gene therapy as used herein refers to the transfer of genetic material (e.g DNA or RNA) of interest into a host to treat or prevent a genetic or acquired
10 disease or condition phenotype. The genetic material of interest encodes a product (e.g. a protein, polypeptide, peptide, functional RNA, antisense) whose production in vivo is desired. For example, the genetic material of interest can encode a hormone, receptor, enzyme,
15 polypeptide or peptide of therapeutic value. Alternatively, the genetic material of interest encodes a suicide gene. For a review see, in general, the text "Gene Therapy" (Advances in Pharmacology 40, Academic Press, 1997).

20 Two basic approaches to gene therapy have evolved: (1) *ex vivo* and (2) *in vivo* gene therapy. In *ex vivo* gene therapy cells are removed from a patient, and while being cultured are treated *in vitro*. Generally, a functional replacement gene is introduced into the cell
25 via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination,

etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the host/patient. These genetically reimplanted cells have been shown to express the transfected genetic material *in situ*.

In *in vivo* gene therapy, target cells are not removed from the subject rather the genetic material to be transferred is introduced into the cells of the recipient organism *in situ*, that is within the recipient.

In an alternative embodiment, if the host gene is defective, the gene is repaired *in situ* [Culver, 1998]. These genetically altered cells have been shown to express the transfected genetic material *in situ*.

The gene expression vehicle is capable of delivery/transfer of heterologous nucleic acid into a host cell. The expression vehicle may include elements to control targeting, expression and transcription of the nucleic acid in a cell selective manner as is known in the art. It should be noted that often the 5'UTR and/or 3'UTR of the gene may be replaced by the 5'UTR and/or 3'UTR of the expression vehicle. Therefore as used herein the expression vehicle may, as needed, not include the 5'UTR and/or 3'UTR of the actual gene to be transferred and only include the specific amino acid coding region.

The expression vehicle can include a promotor for controlling transcription of the heterologous material and can be either a constitutive or inducible promotor to allow selective transcription. Enhancers that may be
5 required to obtain necessary transcription levels can optionally be included. Enhancers are generally any non-translated DNA sequence which works contiguously with the coding sequence (in cis) to change the basal transcription level dictated by the promoter. The
10 expression vehicle can also include a selection gene as described herein below.

Vectors can be introduced into cells or tissues by any one of a variety of known methods within the art. Such methods can be found generally described in Sambrook
15 et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989), Chang et al., *Somatic Gene Therapy*, CRC Press, Ann Arbor, MI
20 (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor, MI (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston MA (1988) and Gilboa et al (1986) and include, for example, stable or transient transfection, lipofection, electroporation
25 and infection with recombinant viral vectors. In addition, see United States patent 4,866,042 for vectors

involving the central nervous system and also United States patents 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by infection offers
5 several advantages over the other listed methods. Higher efficiency can be obtained due to their infectious nature. Moreover, viruses are very specialized and typically infect and propagate in specific cell types. Thus, their natural specificity can be used to target the
10 vectors to specific cell types *in vivo* or within a tissue or mixed culture of cells. Viral vectors can also be modified with specific receptors or ligands to alter target specificity through receptor mediated events.

A specific example of DNA viral vector for
15 introducing and expressing recombinant sequences is the adenovirus derived vector Adenop53TK. This vector expresses a herpes virus thymidine kinase (TK) gene for either positive or negative selection and an expression cassette for desired recombinant sequences. This vector
20 can be used to infect cells that have an adenovirus receptor which includes most cancers of epithelial origin as well as others. This vector as well as others that exhibit similar desired functions can be used to treat a mixed population of cells and can include, for example,
25 an *in vitro* or *ex vivo* culture of cells, a tissue or a human subject.

Additional features can be added to the vector to ensure its safety and/or enhance its therapeutic efficacy. Such features include, for example, markers that can be used to negatively select against cells
5 infected with the recombinant virus. An example of such a negative selection marker is the TK gene described above that confers sensitivity to the antibiotic gancyclovir. Negative selection is therefore a means by which infection can be controlled because it provides
10 inducible suicide through the addition of antibiotic. Such protection ensures that if, for example, mutations arise that produce altered forms of the viral vector or recombinant sequence, cellular transformation will not occur.

15 Features that limit expression to particular cell types can also be included. Such features include, for example, promoter and regulatory elements that are specific for the desired cell type.

In addition, recombinant viral vectors are useful
20 for *in vivo* expression of a desired nucleic acid because they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many
25 progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly

infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can
5 also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

As described above, viruses are very specialized
10 infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural
15 specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. The vector to be used in the methods of the invention will depend on desired cell type to be targeted and will be known to those skilled in the art. For example, if breast cancer is to be treated then a
20 vector specific for such epithelial cells would be used. Likewise, if diseases or pathological conditions of the hematopoietic system are to be treated, then a viral vector that is specific for blood cells and their precursors, preferably for the specific type of
25 hematopoietic cell, would be used.

Retroviral vectors can be constructed to function either as infectious particles or to undergo only a single initial round of infection. In the former case, the genome of the virus is modified so that it maintains
5 all the necessary genes, regulatory sequences and packaging signals to synthesize new viral proteins and RNA. Once these molecules are synthesized, the host cell packages the RNA into new viral particles which are capable of undergoing further rounds of infection. The
10 vector's genome is also engineered to encode and express the desired recombinant gene. In the case of non-infectious viral vectors, the vector genome is usually mutated to destroy the viral packaging signal that is required to encapsulate the RNA into viral particles.
15 Without such a signal, any particles that are formed will not contain a genome and therefore cannot proceed through subsequent rounds of infection. The specific type of vector will depend upon the intended application. The actual vectors are also known and readily available
20 within the art or can be constructed by one skilled in the art using well-known methodology.

The recombinant vector can be administered in several ways. If viral vectors are used, for example, the procedure can take advantage of their target
25 specificity and consequently, do not have to be administered locally at the diseased site. However,

local administration can provide a quicker and more effective treatment, administration can also be performed by, for example, intravenous or subcutaneous injection into the subject. Injection of the viral vectors into a
5 spinal fluid can also be used as a mode of administration, especially in the case of neurodegenerative diseases. Following injection, the viral vectors will circulate until they recognize host cells with the appropriate target specificity for infection.

10 An alternate mode of administration can be by direct inoculation locally at the site of the disease or pathological condition or by inoculation into the vascular system supplying the site with nutrients or into the spinal fluid. Local administration is advantageous
15 because there is no dilution effect and, therefore, a smaller dose is required to achieve expression in a majority of the targeted cells. Additionally, local inoculation can alleviate the targeting requirement required with other forms of administration since a
20 vector can be used that infects all cells in the inoculated area. If expression is desired in only a specific subset of cells within the inoculated area, then promoter and regulatory elements that are specific for the desired subset can be used to accomplish this goal.
25 Such non-targeting vectors can be, for example, viral vectors, viral genome, plasmids, phagemids and the like.

Transfection vehicles such as liposomes can also be used to introduce the non-viral vectors described above into recipient cells within the inoculated area. Such transfection vehicles are known by one skilled within the art.

The pharmaceutical compositions containing the active ingredients of the present invention as described herein above are administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of administration, patient age, sex, body weight and other factors known to medical practitioners. The pharmaceutically "effective amount" for purposes herein is thus determined by such considerations as are known in the medical arts. The amount must be effective to achieve improvement including but not limited to improved survival rate or more rapid recovery, or improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the medical arts. The pharmaceutical compositions can be combinations of the active ingredients but will include at least one active ingredient.

In the method of the present invention, the pharmaceutical compositions of the present invention can be administered in various ways taking into account the

nature of compounds in the pharmaceutical compositions. It should be noted that they can be administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in
5 combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The compounds can be administered orally, subcutaneously or parenterally including intravenous, intraarterial, intramuscular, intraperitoneally, and intranasal administration as well
10 as intrathecal and infusion techniques. Implants of the compounds are also useful. The patient being treated is a warm-blooded animal and, in particular, mammals including man. The pharmaceutically acceptable carriers, diluents, adjuvants and vehicles as well as implant
15 carriers generally refer to inert, non-toxic solid or liquid fillers, diluents or encapsulating material not reacting with the active ingredients of the invention.

It is noted that humans are treated generally longer than the mice or other experimental animals exemplified
20 herein which treatment has a length proportional to the length of the disease process and drug effectiveness. The doses may be single doses or multiple doses over a period of several days, but single doses are preferred.

The doses may be single doses or multiple doses over
25 a period of several days. The treatment generally has a length proportional to the length of the disease process

and drug effectiveness and the patient species being treated.

When administering the compound of the present invention parenterally, it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion). The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such as cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial

and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Sterile injectable solutions can be prepared by incorporating the compounds utilized in practicing the present invention in the required amount of the appropriate solvent with various of the other ingredients, as desired.

A pharmacological formulation of the present invention can be administered to the patient in an injectable formulation containing any compatible carrier, such as various vehicle, adjuvants, additives, and diluents; or the compounds utilized in the present invention can be administered parenterally to the patient in the form of slow-release subcutaneous implants or targeted delivery systems such as monoclonal antibodies, vectored delivery, iontophoretic, polymer matrices, liposomes, and microspheres. Examples of delivery systems useful in the present invention include:

5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678;
4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196;
and 4,475,196. Many other such implants, delivery
systems, and modules are well known to those skilled in
5 the art.

A pharmacological formulation of the compound
utilized in the present invention can be administered
orally to the patient. Conventional methods such as
administering the compounds in tablets, suspensions,
10 solutions, emulsions, capsules, powders, syrups and the
like are usable. Known techniques which deliver it
orally or intravenously and retain the biological
activity are preferred.

In one embodiment, the compound of the present
15 invention can be administered initially by intravenous
injection to bring blood levels to a suitable level. The
patient's levels are then maintained by an oral dosage
form, although other forms of administration, dependent
upon the patient's condition and as indicated above, can
20 be used. The quantity to be administered will vary for
the patient being treated and will vary from about 100
ng/kg of body weight to 100 mg/kg of body weight per day
and preferably will be from 10 μ g/kg to 10 mg/kg per day.

The present invention also provides a method of
25 diagnosing the presence of ischemia in a patient
including the steps of analyzing a bodily fluid or tissue

sample from the patient for the presence or gene product of at least one expressed gene (up-regulated) as set forth in the group comprising SEQ ID No:1; SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 or

5 proteins as set forth in SEQ ID Nos:7-11 and where ischemia is determined if the up-regulated gene or gene product is ascertained as described herein in the Example. The bodily fluids may include tears, serum, urine, sweat or other bodily fluid where secreted

10 proteins from the tissue that is undergoing an ischemic event may be localized. Additional methods for identification of the gene or gene product are immunoassays, such as and ELISA or radioimmunoassays (RIA), can be used as are known to those in the art

15 particularly to identify gene products in the samples. Immunohistochemical staining of tissue samples is also utilized for identification. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents

20 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521. Further for identification of the gene, in situ hybridization, Southern blotting, single strand

25 conformational polymorphism, restriction endonuclease fingerprinting (REF), PCR amplification and DNA-chip

analysis using nucleic acid sequence of the present invention as primers can be used.

The above discussion provides a factual basis for the use of genes to regulate hypoxia and ischemia and thereby also apoptosis and angiogenesis. The methods used with and the utility of the present invention can be shown by the following non-limiting example and accompanying figures.

10

EXAMPLE**METHODS:**

Most of the techniques used in molecular biology are widely practiced in the art, and most practitioners are familiar with the standard resource materials which describe specific conditions and procedures. However, for convenience, the following paragraphs may serve as a guideline.

General methods in molecular biology: Standard molecular biology techniques known in the art and not specifically described were generally followed as in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989), and in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Maryland (1989) particularly for the Northern Analysis and In Situ analysis and in Perbal, *A Practical Guide to Molecular*

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Cloning, John Wiley & Sons, New York (1988), and in
Watson et al., *Recombinant DNA*, Scientific American
Books, New York. Polymerase chain reaction (PCR) was
carried out generally as in *PCR Protocols: A Guide To*
5 *Methods And Applications*, Academic Press, San Diego, CA
(1990).

Reactions and manipulations involving other nucleic
acid techniques, unless stated otherwise, were performed
as generally described in Sambrook et al., 1989,
10 *Molecular Cloning: A Laboratory Manual*, Cold Spring
Harbor Laboratory Press, and methodology as set forth in
United States patents 4,666,828; 4,683,202; 4,801,531;
5,192,659 and 5,272,057 and incorporated herein by
reference.

15 Additionally, In situ (In cell) PCR in combination
with flow cytometry can be used for detection of cells
containing specific DNA and mRNA sequences (Testoni et
al, 1996, Blood 87:3822).

General methods in immunology: Standard methods in
20 immunology known in the art and not specifically
described are generally followed as in Stites et
al.(eds), *Basic and Clinical Immunology* (8th Edition),
Appleton & Lange, Norwalk, CT (1994) and Mishell and
Shiigi (eds), *Selected Methods in Cellular Immunology*,
25 W.H. Freeman and Co., New York (1980). Available
immunoassays are extensively described in the patent and

scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989.

Differential Analysis

For example C6 glioma cells or other appropriate cells, cell lines or tissues are grown under normal conditions (Normoxia) or under oxygen deprivation conditions (Hypoxia) generally for four to sixteen hours. The cells are harvested and RNA is prepared from the cytoplasmic extracts and from the nuclear fractions. Following the extraction of RNA, fluorescent cDNA probes are prepared. Each condition (for example 4 hours hypoxia and normoxia) is labeled with a different fluorescent dye. For example a probe can be composed of a mixture of Cy3 -dCTP cDNA prepared from RNA extracted from hypoxic cells and with Cy5-dCTP cDNA prepared from RNA extracted from normoxic cells. The probes are used for hybridization to micro-array containing individually spotted cDNA clones derived from C6 cells that were exposed to hypoxia. Differential expression is measured by the amount of fluorescent cDNA that hybridizes to each of the clones on the array. Genes that are up regulated

under hypoxia will have more fluorescence of Cy3 than Cy5. The results show genes that are transcriptionally induced mRNA species that respond very fast to hypoxia.

Differential display:

- 5 Reverse transcription: 2 μ g of RNA are annealed with 1pmol of oligo dT primer (dT)₁₈ in a volume of 6.5 μ l by heating to 70°C for five minutes and cooling on ice. 2 μ l reaction buffer (x5), 1 μ l of 10mM dNTP mix, and 0.5 μ l of SuperScript II reverse transcriptase (GibcoBRL) is added.
- 10 The reaction is carried for one hour at 42°C. The reaction is stopped by adding 70 μ l TE (10mM Tris pH=8; 0.1mM EDTA).
- Oligonucleotides used for Differential display: The oligonucleotides are generally those described in the
- 15 Delta RNA Fingerprinting kit (Clonetech Labs. Inc.).
- Amplification reactions: Each reaction is done in 20 μ l and contains 50 μ M dNTP mix, 1 μ M from each primer, 1x polymerase buffer, 1 unit expand Polymerase (Beohringer Mannheim), 2 μ Ci [α -³²P]dATP and 1 μ l cDNA template.
- 20 Cycling conditions are generally: three minutes at 95°C, then three cycles of two minutes at 94°C, five minutes at 40°C, five minutes at 68°C. This is followed by 27 cycles of one minute at 94°C, two minutes at 60°C, two minutes at 68°C. Reactions were terminated by a seven minute
- 25 incubation at 68°C and addition of 20 μ l sequencing stop

solution (95% formamide, 10mM NaOH, 0.025% bromophenol blue, 0.025% xylene cyanol).

Gel analysis: Generally 3-4 μ l are loaded onto a 5% sequencing polyacrylamide gel and samples are
5 electrophoresed at 2000 volts/40 milliamperes until the slow dye (xylene cyanol) is about 2 cm from the bottom. The gel is transferred to a filter paper, dried under vacuum and exposed to x-ray film.

Recovery of differential bands: Bands showing any a
10 differential between the various pools are excised out of the dried gel and placed in a microcentrifuge tube. 50 μ l of sterile H₂O are added and the tubes heated to 100°C for five minutes. 1 μ l is added to a 49 μ l PCR reaction using the same primers used for the differential display and
15 the samples are amplified for 30 cycles of: one minute at 94°C, one minute at 60°C and one minute at 68°C. 10 μ l is analyzed on agarous gel to visualize and confirm successful amplification.

Representational difference analysis

20 Reverse transcription: as above but with 2 μ g polyA+ selected mRNA.

Preparation of double stranded cDNA: cDNA from the previous step is treated with alkali to remove the mRNA, precipitated and dissolved in 20 μ l H₂O. 5 μ l buffer, 2 μ l
25 10mM dATP, H₂O to 48 μ l and 2 μ l terminal deoxynucleotide transferase (TdT) are added. The reaction is incubated

2-4 hours at 37°C. 5µl oligo dT (1µg/µl) was added and incubated at 60°C for five minutes. 5µl 200 mM DTT, 10 µl 10x section buffer (100mM Mg Cl₂, 900 mM Hepes, pH 6.6) 16 µl dNTPs (1 mM), and 16 U of Klenow are added and the
5 mixture incubated overnight at room temperature to generate ds cDNA. 100µl TE is added and extracted with phenol/chloroform. The DNA is precipitated and dissolved in 50µl H₂O.

Generation of representations: cDNA with DpnII is
10 digested by adding 3µl DpnII reaction buffer 20 V and DpnII to 25µl cDNA and incubated five hours at 37°C. 50µl TE is added and extracted with phenol/chloroform. cDNA is precipitated and dissolved to a concentration of 10ng/µl.

15 Driver: 1.2µg DpnII digested cDNA. 4µl from each oligo and 5µl ligation buffer x10 and annealed at 60°C for ten minutes. 2µl ligase is added and incubated overnight at 16°C. The ligation mixture is diluted by adding 140µl TE. Amplification is carried out in a volume of 200µl using
20 appropriate primer and 2µl ligation product and repeated in twenty tubes for each sample. Before adding Taq DNA polymerase, the tubes are heated to 72°C for three minutes. PCR conditions are as follows: five minutes at 72°C, twenty cycles of one minute at 95°C and three
25 minutes at 72°C, followed by ten minutes at 72°C.

Every four reactions were combined, extracted with phenol/chloroform and precipitated. Amplified DNA is dissolved to a concentration of $0.5\mu\text{g}/\mu\text{l}$ and all samples are pooled.

- 5 Subtraction: Tester DNA ($20\mu\text{g}$) is digested with DpnII as above and separated on a 1.2% agarous gel. The DNA is extracted from the gel and $2\mu\text{g}$ ligated to the appropriate oligos. The ligated Tester DNA is then diluted to $10\text{ng}/\mu\text{l}$ with TE. Driver DNA is digested with DpnII and
- 10 repurified to a final concentration of $0.5\mu\text{g}/\mu\text{l}$. Mix $40\mu\text{g}$ of Driver DNA with $0.4\mu\text{g}$ of Tester DNA. Extraction is carried out with phenol/chloroform and precipitated using two washes with 70% ethanol, resuspended DNA in $4\mu\text{l}$ of 30mM EPPS pH=8.0, 3mM EDTA and overlayed with $35\mu\text{l}$
- 15 mineral oil. Denature at 98°C for five minutes, cool to 67°C and $1\mu\text{l}$ of 5M NaCl added to the DNA. Incubate at 67°C for twenty hours. Dilute DNA by adding $400\mu\text{l}$ TE.
- 20 Amplification: Amplification of subtracted DNA in a final volume of $200\mu\text{l}$ as follows: Buffer, nucleotides and $20\mu\text{l}$ of the diluted DNA are added, heated to 72°C , and Taq DNA polymerase added. Incubate at 72°C for five minutes and add appropriate oligo. Ten cycles of one minute at 95°C , three minutes at 70°C are performed. Incubate ten minutes at 72°C . The amplification is
- 25 repeated in four separate tubes. The amplified DNA is extracted with phenol/chloroform, precipitated and all

four tubes combined in 40 μ l 0.2xTE, and digested with Mung Bean Nuclease as follows: To 20 μ l DNA 4 μ l buffer, 14 μ l H₂O and 2 μ l Mung Bean Nuclease (10 units/ μ l) added. Incubate at 30°C for thirty-five minutes + First

5 Differential Product (DPI).

Repeat subtraction hybridization and PCR amplification at driver: differential ratio of 1:400 (DPII) and 1:40,000 (DPIII) using appropriate oligonucleotides. Differential products are then cloned into a Bluescript vector at the

10 BAM HI site for analysis of the individual clones.

DIFFERENTIAL EXPRESSION USING GENE EXPRESSION MICRO-ARRAY

Messenger RNA isolated as described herein above is labeled with fluorescent dNTP's using a reverse

15 transcription reaction to generate a labeled cDNA probe. mRNA is extracted from C6 cells cultured in normoxia conditions and labeled with Cy3-dCTP (Amersham) and mRNA extracted from C6 cells cultured under hypoxia conditions is labeled with Cy5-dCTP (Amersham). The two labeled

20 cDNA probes are then mixed and hybridized onto a microarray (Schena et al, 1996) composed of for example 2000 cDNA clones derived from a cDNA library prepared from C6 cells cultured under hypoxic conditions.

Following hybridization the microarray is scanned using

25 a laser scanner and amount of fluorescence of each of the fluorescence dyes is measured for each cDNA clone on the

micro-array giving an indication of the level of mRNA in each of the original mRNA populations being tested. Comparison of the fluorescence on each cDNA clone on the micro-array between the two different fluorescent dyes is a measure for the differential expression of the indicated genes between the two experimental conditions.

IN SITU ANALYSIS

In situ analysis is performed for the candidate genes identified by the differential response to exposure to hypoxia conditions as described above. The expression is studied in two experimental systems: solid tumors and hypoxic retina.

Solid tumors are formed by injections in mice of the original glioma cells used for the differential expression. The glioma cells form tumors which are then excised, slided and used to individually measure expression levels of the candidate gene. The solid tumor model (Benjamin et al, 1997) shows that the candidate gene's expression is activated in tumors around the hypoxic regions that are found in the center of the tumor and are therefore hypoxia-regulated *in vivo*. Up regulation indicates further that the up-regulated gene can promote angiogenesis that is required to sustain tumor growth.

The hypoxia retina model measures expression levels in an organ that is exposed to hypoxia (ischemia) and directly mimics retinopathy. Hypoxia in the retina is created by exposing new born rats to hyperoxia which
5 diminishes blood vessels in the retinas (Alon et al., 1995). Upon transfer to normal oxygen levels, relative hypoxia is formed due to the lack of blood supply. The hypoxic retina is excised, sliced and used to monitor the expression of the candidate genes.

10

RESULTS

Utilizing gene expression microarray analysis the genes set forth in SEQ ID Nos:1-6 were identified as being differentially expressed under hypoxia conditions.

15 As shown in the figures differential expression under hypoxia conditions was observed. Northern Analysis was performed with 32P-dCTP labeled probes derived from the candidate genes. Two micrograms of mRNA were fractionated on formaldehyde containing agarose gels,
20 blotted onto a nitrocellulose membrane and hybridized to the labeled cDNA probes. To monitor the kinetics of expression as a result of hypoxia, mRNA was prepared from cells in normoxia, and 4 and 16 hours exposure to hypoxia conditions. The results of the analysis showed that all
25 the genes (SEQ ID Nos:1-6) were induced by hypoxic

conditions, confirming the results obtained by the gene expression microarray analysis.

In the *in situ* analysis using the solid tumor model SEQ ID Nos:1-6 were upregulated, that is expressed. In
5 the retina model SEQ ID Nos:1, 2 and 6 were found to be upregulated in this model.

SEQ ID No:1 (RTP801) is the rat homolog of SEQ ID No:2 (RTP779). The protein sequences are SEQ ID No:9 and SEQ ID No:10 respectively. Both of these genes have not
10 been reported in gene data bases and are expressed under hypoxic stress and are up-regulated in both of the *in situ* analyses. The expression of this gene was observed in the ovary where active apoptosis was occurring. Its regulation is HIF-1 dependent (Carmeliet et al, 1998)
15 indicating further that the gene is associated with hypoxia-induced apoptosis. Some homology was found between the 3'UTR of RTP801 and the 5'UTR of a transcription factor (rat) pet-1 (Carmeliet et al, 1998; Spence et al, 1998; Fyodorov et al, 1998).

20 SEQ ID No:3 (RTP241) is 1902 bp long, has not been reported in gene data bases and is expressed under hypoxic stress and up-regulated in both *in situ* analyses. The gene sequence has some homology with a yeast gene located upstream to the cox14 gene. The protein (SEQ ID
25 No:7) coded by the sequence contains a signal peptide region and therefore is secreted.

SEQ ID No:4 (RTP220) is 4719 bp long, has not been reported in gene data bases and is expressed under hypoxic stress and up-regulated in the tumor *in situ* analysis. The gene sequence has some homology with annilin from Drosophila. The protein sequence is set forth in SEQ ID No:11.

SEQ ID No:5 (RTP953/359) is a partial gene sequence that has not been found in gene data bases and is expressed under hypoxic stress and up-regulated in both *in situ* analyses.

SEQ ID No:6 (RTP971) is expressed under hypoxic stress and up-regulated in the tumor *in situ* analysis. The original analysis used the rat sequence. SEQ ID No:6 is the human homolog and has greater than 90% homology with the rat sequence. Based on preliminary sequence analysis it appears to be the gene Neuroleukin or a member of that gene family. The gene has not been reported to be responsive to hypoxia conditions and is reported to be a new motility factor for astrocytes. The reported gene encodes a protein (SEQ ID No:8, human homolog) that is identified as a glycolytic enzyme phosphohexose isomerase and as a survival factor for neurons (Niinaka et al, 1998; Watanabe et al., 1996).

Astrocyte motility is an important factor in the formation of blood vessels (angiogenesis) in brain and retina. Astrocytes can be considered oxygen level

sensors as they respond under hypoxic conditions by secretion of angiogenic factors like WEGF. In an experiment primary astrocyte cultures were established and grown in vitro without serum and the astrocytes were immobile. However when conditioned medium from retinal cultures cultured under hypoxic conditions was added to the astrocyte cultures motility was observed. If the neuroleukin inhibitor (Obese et al., 1990), D-erythrose 4-phosphate (at 1.25mM) was added clear indications of inhibition of motility were observed in the astrocyte cultures indicating that the astrocyte motility (and stellation) was dependent on neuroleukin activity. Other results show that SEQ ID No:6 is also HIF-1 dependent indicating further that the gene is associated with hypoxia-induced angiogenesis and apoptosis.

Throughout this application, various publications, including United States patents, are referenced by author and year and patents by number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology

which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above
5 teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described.

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CLAIMS

What is claimed is:

1. A purified, isolated and cloned nucleic acid sequences encoding hypoxia-regulated genes which have sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, and SEQ ID No:5 or having a complementary or allelic variation sequence thereto.
2. A purified, isolated and cloned nucleic acid sequence according to claim 1 wherein the nucleic acid is mRNA.
3. A purified protein as encoded by a nucleic acid selected from the nucleic acid sequences as set forth in claim 1.
4. An antibody which specifically binds to the protein of claim 3.
5. An antibody of claim 4 selected from the group consisting of monoclonal and polyclonal antibody.
6. An antibody of claim 5 conjugated to a detectable moiety.
7. A non-human transgenic mammal or cell lines containing an expressible nucleic acid sequence selected from the nucleic acid sequences set forth in claim 1.
8. A non-human eucaryotic organism in which an equivalent nucleic acid sequence selected from the nucleic acid sequences of claim 1 is knocked out.
9. A purified, isolated and cloned nucleic acid sequences as set forth in claim 1 which has the sequence as set forth in SEQ ID No:1.
10. A purified, isolated and cloned nucleic acid sequences as set forth in claim 1 which has the sequence as set forth in SEQ ID No:2.
11. A purified, isolated and cloned nucleic acid sequences as set forth in claim 1 which has the sequence as set forth in SEQ ID No:3.

12. A purified, isolated and cloned nucleic acid sequences as set forth in claim 1 which has the sequence as set forth in SEQ ID No:4.

13. A purified, isolated and cloned nucleic acid sequences as set forth in claim 1 which has the sequence as set forth in SEQ ID No:5.

14. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of a protein selected from the group consisting of SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

15. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a dominant negative peptide directed against at least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 or the proteins encoded thereof.

16. A method of regulating angiogenesis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

17. A method of providing an apoptotic regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

18. A method of providing an angiogenesis regulating gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

19. A method of regulating response to hypoxia conditions in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at

least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

20. A method of providing a hypoxia regulated gene by administering directly to a patient in need of such therapy an expressible vector comprising expression control sequences operably linked to one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

21. A method of diagnosing the presence of ischemia in a patient including the steps of analyzing a sample from the patient for the presence of at least one expressed gene as set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

22. The method as set forth in claim 21 wherein the sample is a bodily fluid sample and the presence of at least one protein encoded by SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 is ascertained indicating the presence of ischemia.

23. The method as set forth in claim 21 wherein the sample is a tissue sample and the presence of at least one up-regulated gene encoded by SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 is ascertained indicating the presence of ischemia.

24. A purified, isolated and cloned nucleic acid sequences having angiogenesis regulating activity which have sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 or having a complementary or allelic variation sequence thereto.

25. A purified, isolated and cloned nucleic acid sequences having apoptosis regulating activity which have sequences as set forth in the group comprising SEQ ID No:1, SEQ ID No:2, SEQ ID No:3, SEQ ID No:4, SEQ ID No:5 and SEQ ID No:6 or having a complementary or allelic variation sequence thereto.

26. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antagonist of a

protein selected from the group consisting of SEQ ID No:7, SEQ ID No:8, SEQ ID No:9, SEQ ID No:10 and SEQ ID No:11.

27. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of a dominant negative peptide directed against at least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6 or ther proteins encoded thereof.

28. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of an antisense oligonucleotide directed against at least one of the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

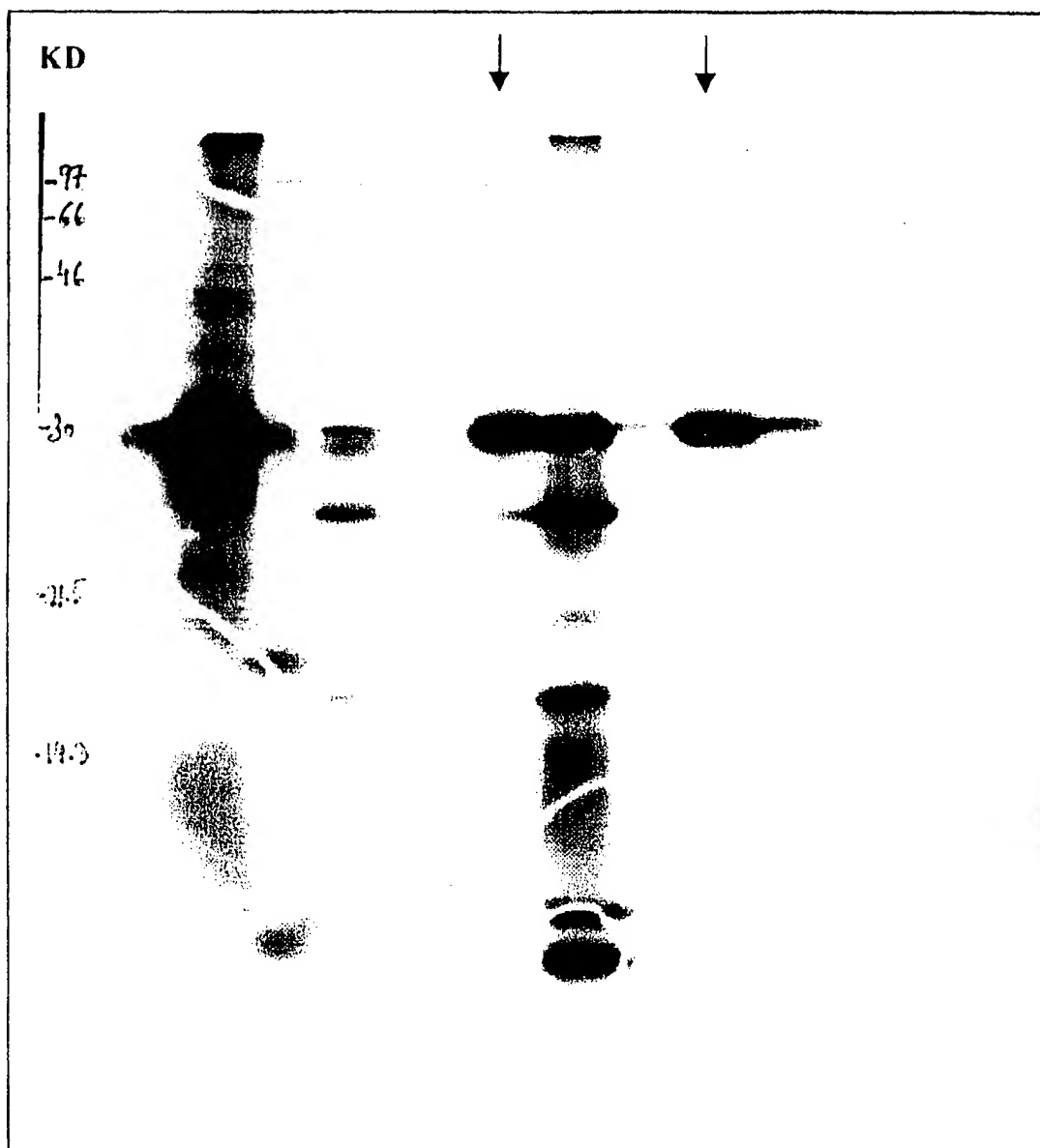
29. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one protein encoded by the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

30. The method as set forth in claim 28 wherein the protein has a sequence selected from the group comprising SEQ ID No:7, SEQ ID No:8, SEQ ID No:10 and SEQ ID No:11

31. A method of regulating apoptosis in a patient in need of such treatment by administering to a patient a therapeutically effective amount of at least one protein encoded by the sequences set forth in the group comprising SEQ ID No:2; SEQ ID No:3; SEQ ID No:4; SEQ ID No:5; and SEQ ID No:6.

32. The method as set forth in claim 30 wherein the protein has a sequence selected from the group comprising SEQ ID No:7, SEQ ID No:8, SEQ ID No:10 and SEQ ID No:11

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Fig-1

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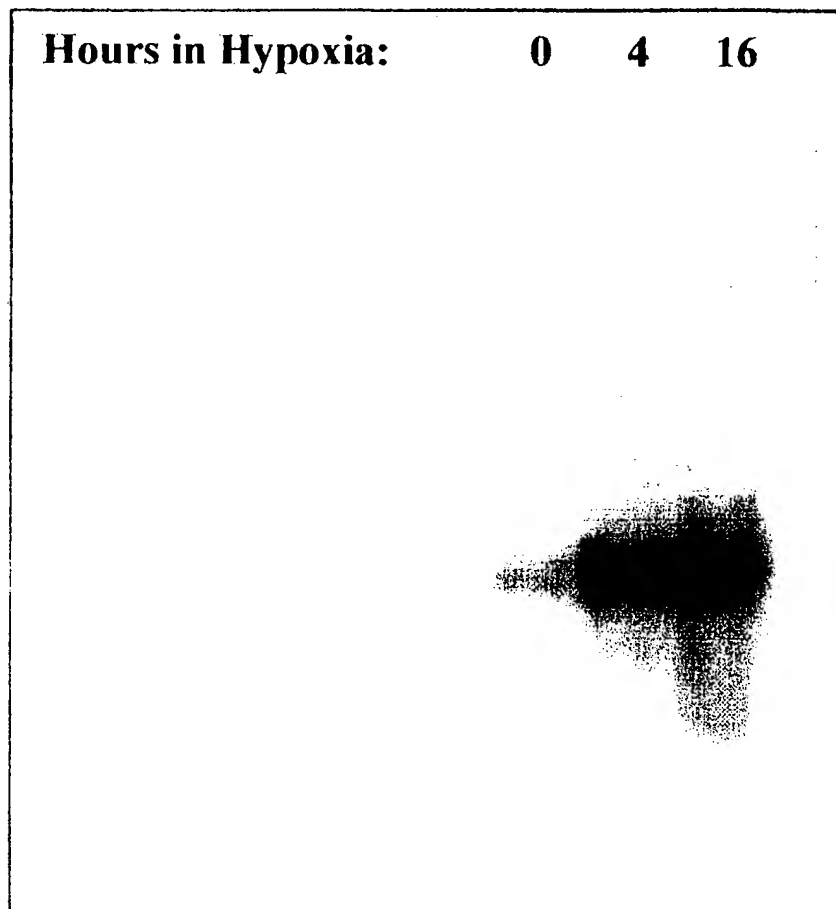


Fig-2

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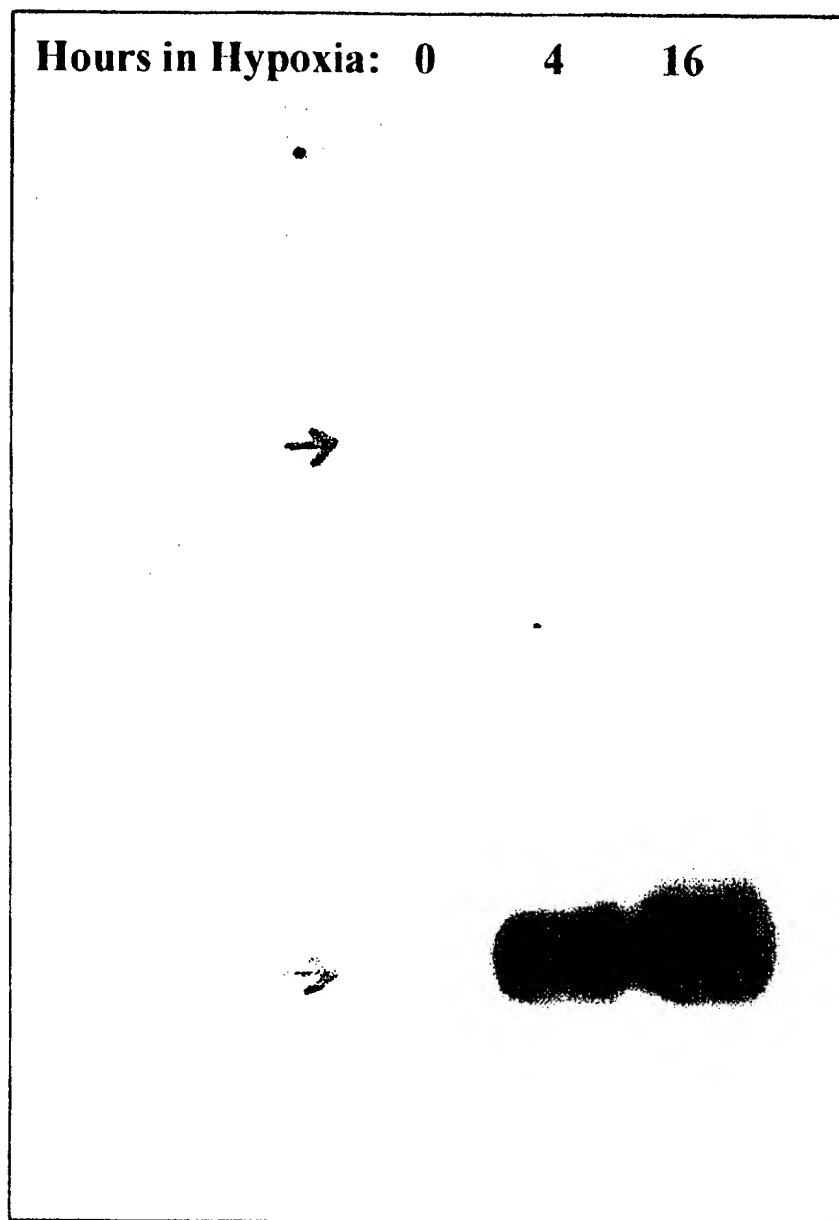
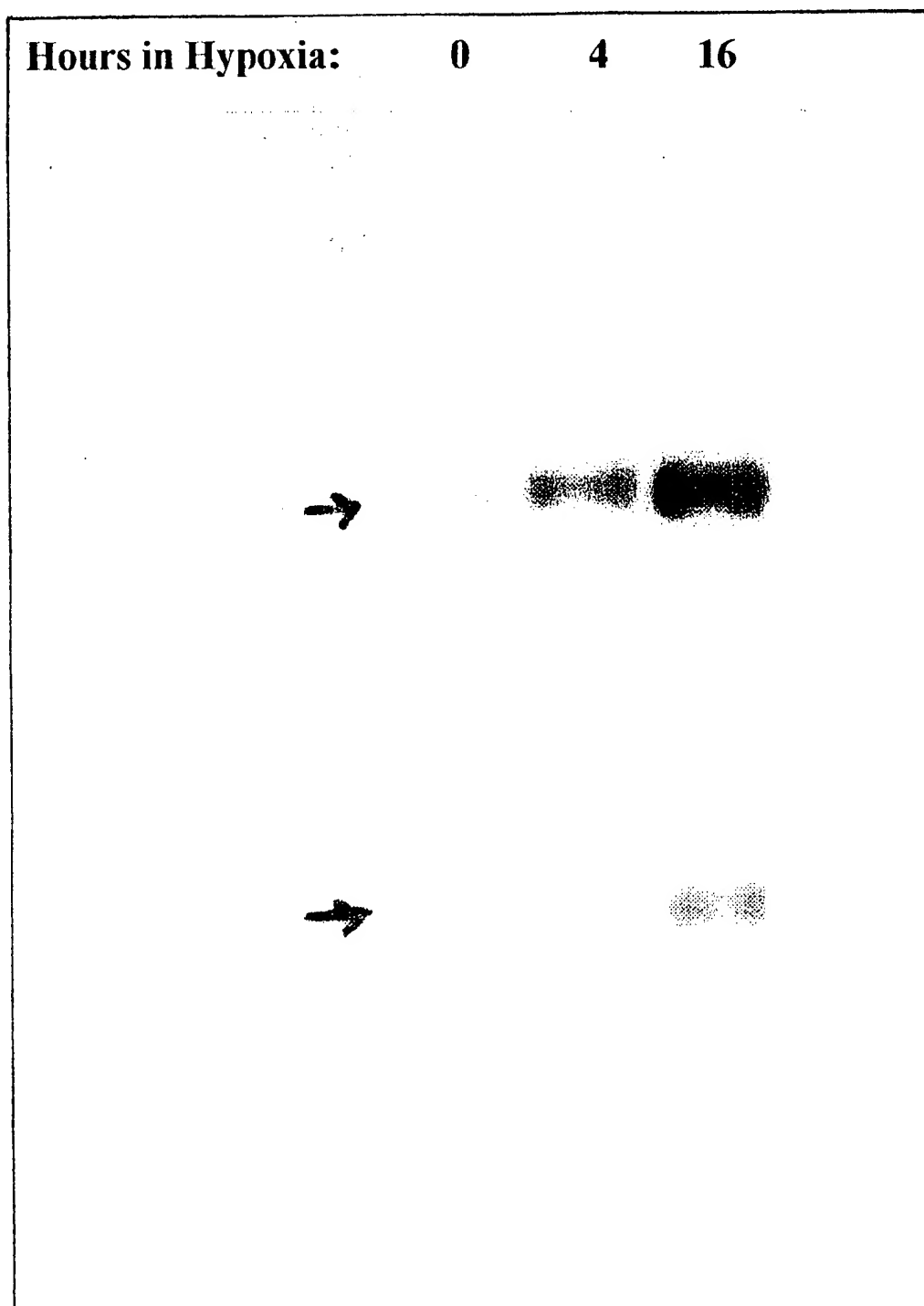
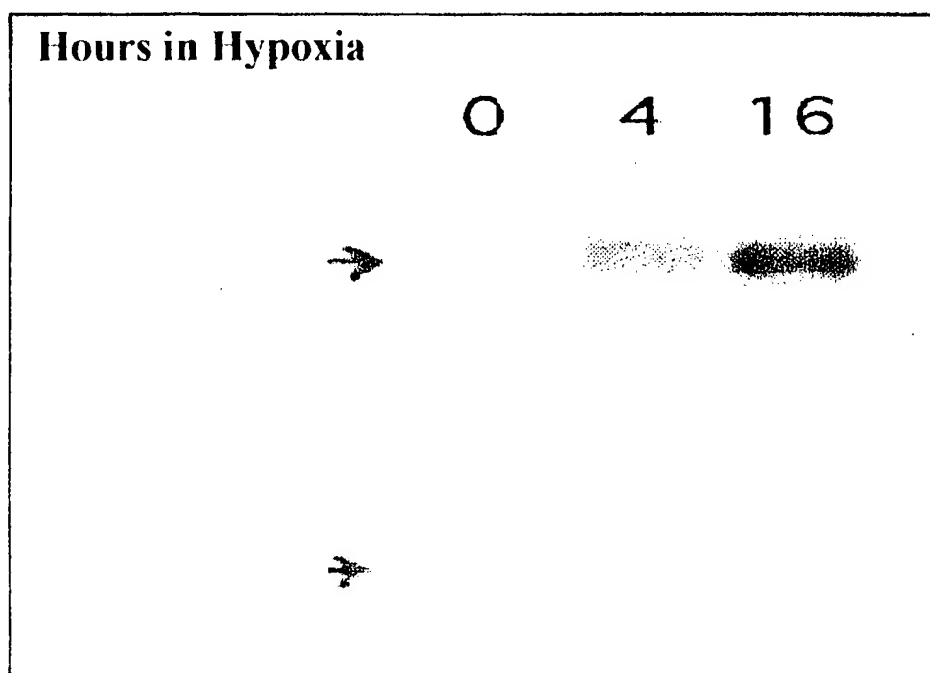


Fig-3

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*Fig-4*

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Fig-5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Einat, Paz
Skaliter, Rami
- (ii) TITLE OF INVENTION: HYPOXIA-REGULATED GENES
- (iii) NUMBER OF SEQUENCES: 11
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: KOHN & ASSOCIATES
 - (B) STREET: 30500 Northwestern Hwy., Suite 401
 - (C) CITY: Farmington Hills
 - (D) STATE: Michigan
 - (E) COUNTRY: U.S.
 - (F) ZIP: 48334
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Kohn, Kenneth I.
 - (B) REGISTRATION NUMBER: 30,955
 - (C) REFERENCE/DOCKET NUMBER: 0168.00038
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (248) 539-5050
 - (B) TELEFAX: (248) 5395055

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1754 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GCTAGCTGCG GCTTCTGTGC TCCTTCGCCG AACCTCATCA ACCAGCGTCC TGGCGTCTGA	180
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AGGACTGTCC CCTGTGTTAG AAAACTGACA ATAGCCACCG GAGGGGCGCA GGGCCAGGTG 1020
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1782 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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TGAGTTCATC AGCAAACGCC CTGGCGTCTG TCCTCACCAT GCCTAGCCTT TGGGACCGCT	240
TCTCGTCGTC GTCCACCTCC TCTTCGCCCT CGTCCTTGCC CCGAACTCCC ACCCCAGATC	300
GGCCGCCGCG CTCAGCCTGG GGGTCGGCGA CCCGGGAGGA GGGGTTTGAC CGCTCCACGA	360
GCCTGGAGAG CTCGGACTGC GAGTCCCTGG ACAGCAGCAA CAGTGGCTTC GGGCCGGAGG	420
AAGACACGGC TTACCTGGAT GGGGTGTCGT TGCCCGACTT CGAGCTGCTC AGTGACCCTG	480
AGGATGAACA CTTGTGTGCC AACCTGATGC AGCTGCTGCA GGAGAGCCTG GCCCAGGCGC	540
GGCTGGGCTC TCGACGCCCT GCGCGCCTGC TGATGCCTAG CCAGTTGGTA AGCCAGGTGG	600
GCAAAGAACT ACTGCGCCTG GCCTACAGCG AGCCGTGCGG CCTGCGGGGG GCGCTGCTGG	660
ACGTCTGCGT GGAGCAGGGC AAGAGCTGCC ACAGCGTGGG CCAGCTGGCA CTCGACCCCA	720
GCCTGGTGCC CACCTTCCAG CTGACCCTCG TGCTGCGCCT GGA CTCACGA CTCTGGCCCA	780
AGATCCAGGG GCTGTTTAGC TCCGCCAACT CTCCCTTCCT CCCTGGCTTC AGCCAGTCCC	840
TGACGCTGAG CACTGGCTTC CGAGTCATCA AGAAGAAGCT GTACAGCTCG GAACAGCTGC	900
TCATTGAGGA GTGTTGAACT TCAACCTGAG GGGGCCGACA GTGCCCTCCA AGACAGAGAC	960
GA CTGAACTT TTGGGGTGGG GACTAGAGGC AGGAGCTGAG GGA CTGATTC CTGTGGTTGG	1020
AAA ACTGAGG CAGCCACCTA AGGTGGAGGT GGGGGAATAG TGTTTCCCAG GAAGCTCATT	1080
GAGTTGTGTG CGGGTGGCTG TGCATTGGGG ACACATACCC CTCAGTACTG TAGCATGAAA	1140
CAAAGGCTTA GGGGCCAACA AGGCTTCCAG CTGGATGTGT GTGTAGCATG TACCTTATTA	1200
TTTTTGTTAC TGACAGTTAA CAGTGGTGTG ACATCCAGAG AGCAGCTGGG CTGCTCCCGC	1260
CCCAGCCCGG CCCAGGGTGA AGGAAGAGGC ACGTGCTCCT CAGAGCAGCC GGAGGGAGGG	1320
GGGAGGTCGG AGGTGCTGGA GGTGGTTTGT GTATCTTACT GGTCTGAAGG GACCAAGTGT	1380
GTTTGTGTGT TGTTTTGTAT CTTGTTTTTC TGATCGGAGC ATCACTACTG ACCTGTTGTA	1440
GGCAGCTATC TTACAGACGC ATGAATGTAA GAGTAGGAAG GGGTGGGTGT CAGGGATCAC	1500
TTGGGATCTT TGACACTTGA AAAATTACAC CTGGCAGCTG CGTTTAAGCC TTCCCCATC	1560
GTGTACTGCA GAGTTGAGCT GGCAGGGGAG GGGCTGAGAG GGTGGGGGCT GGAACCCCTC	1620
CCCGGGAGGA GTGCCATCTG GGTCTTCCAT CTAGAACTGT TTACATGAAG ATAAGATACT	1680
CACTGTTTCA GAATACACTT GATGTTCAAG TATTAAGACC TATGCAATAT TTTTACTTT	1740
TCTAATAAAC ATGTTTGTTA AAACAAAAAA AAAAAAAAAA AA	1782

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1900 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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CCATCCCTCA TAGGACTAAT TATAGGGTTG GGGGGGCCGC CCCCCCAGGT TCGAGTGGCG      60
ATGGGCCCGCG GCTGGGGCTT GCTCGTCGGA CTCTTGGGCG TCGTGTGGCT GCTGCGGTTCG      120
GGCCAGGGCG AGGAGCAGCA GCAGGAGACA GCGGCACAGC GGTGTTTCTG TCAGGTTAGT      180
GGTTACCTGG ATGACTGTAC CTGTGATGTC GAGACCATCG ATAAGTTTAA TAACTACAGA      240
CTTTTCCCAA GACTACAAAA GCTCCTTGAA AGTGACTACT TTAGATACTA CAAGGTAAAC      300
TTGAGGAAGC CATGTCCTTT CTGGAATGAC ATCAACCAAT GTGGAAGAAG AGACTGTGCT      360
GTCAAACCCT GCCATTCTGA TGAAGTCCCT GATGGAATTA AGTCTGCGAG CTACAAGTAT      420
TCCAAGGAAG CCAACCTCCT TGAGGAGTGT GAGCAGGCTG AGCGGCTCGG AGCAGTGGAC      480
GAATCTCTGA GTGAGGAGAC CCAGAAGGCT GTTCTTCAGT GGACGAAACA CGATGATTCT      540
TCAGACAGCT TCTGTGAAGT TGATGACATA CAGTCCCCCG ATGCTGAGTA TGTGGATTTA      600
CTCCTTAACC CTGAGCGCTA CACAGGCTAC AAGGGGCCGG ACGCTTGGAG GATATGGAGT      660
GTCATCTATG AAGAAAACCTG CTTTAAGCCA CAGACAATTC AAAGGCCTTT GGCTTCGGGG      720
CAAGGAAAAC ATAAAGAGAA CACATTTTAC AGCTGGCTAG AAGGCCTCTG TGTAGAAAAG      780
AGAGCATTCT ACAGGCTTAT ATCTGGCCTA CACGCAAGCA TCAATGTACA TTTGAGTGCA      840
AGGTATCTTT TACAAGATAA TTGGCTGGAA AAGAAATGGG GTCATAATGT CACAGAGTTT      900
CAGCAGCGCT TTGATGGGGT TTTGACAGAA GGAGAAGGCC CCAGGAGGCT GAAGAACCTG      960
TACTTTCTTT ACCTGATAGA GTTAAGGGCT CTCTCTAAAG TGCTTCCGTT TTTCGAGCGC     1020
CCAGATTTTC AGCTCTTCAC TGGAAATAAA GTTCAGGATG TGGAAAACAA AGAGTTACTT     1080
CTGGAGATTC TTCATGAAGT CAAGTCATTT CCTTTGCATT TTGATGAGAA TTCTTTTTTT     1140
GCGGGGGATA AAAACGAAGC ACATAAGCTA AAGGAGGACT TCCGCCTACA CTTTAGAAAC     1200
ATCTCGAGGA TCATGGACTG CGTCGGCTGC TTCAAGTGCC GCCTGTGGGG CAAGCTTCAG     1260
ACTCAGGGTC TGGGCACTGC TCTGAAGATC TTGTTTTCTG AAAAACTGAT CGCAAATATG     1320
CCCGAAAGCG GACCCAGTTA TGAATTCCAG CTAACCAGAC AAGAAATAGT GTCGTTGTTC     1380
AATGCATTCG GAAGGATTTT CACAAGTGTG AGAGAATTAG AGAACTTCAG ACACTTGTTA     1440
CAGAATGTTT ACTGAGGAGG GCGGCTGGAA CCTGCTTGTT TCTGCACAGG GGAGTCCAGA     1500

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GGGCAGAATG TCTGAGCAGC GTGATTGCAG TGACCGTCCT GAGCCAAACG TTCATATCAA	1560
GCTGCCTTTG TCAAAGGAGA GATACATTGT TTTAAGTAAA TGACATTTTT AAACATTGTG	1620
TTCATGTTTA ATATTATTGT GAATAAAAGT AGTATTTTGG TAATGTACAA ATTTTAATAC	1680
TAAGCAAAAG TAAGGTCATT AAATTGCCCT ATGATGGGGT TGGGGATTTA GCTCAGTGGT	1740
AGAGCTCTTG CCTAGGAAGC GCAAGGCCCT GGGTTCGGTC CCCAGCTCCG AAAAAAAGA	1800
ACCCCCCCCC CAAAAAAAT TGCCCCATA AAAAGGGTAG GTGAATCCTG CCCCAGGCTC	1860
TCCACCTAAA TTTTTTTTTG AAAACTTTTT TCCCCAAGG	1900

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4121 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

RTTTTTTTTT CCTTTNAAA NGGNNAAGN NTTCCCCCN CCTTCCTCN ANTAAAAAT	60
TTGGNANCCC AAAANGCTTN GGGGGGCNNN GGGNNCCNT NGGGGNTTGG GGAGTTNCNC	120
CNGGNGANNT TTNCAAGNAA NTTAAANATT TTTTCACCCA ATCNCNNTTT TGGGGAAAAG	180
CCTTGCCTTC ACCTTTCCAA AGCCAACCCG TTTTCAAAGG CTTCAGGTAC CCCCAGTTGG	240
GGAGAAGGGG CCTTTCTGGC CAACCCTTGC TGGCAAACGA TTTGGTTCTT GGAAGATGA	300
TGTTAAGCTA ATTCATTCTG CCAAAGCCAA AATAGTGTA CAAGAACAGC CTGGTACCGG	360
CTTGTTTATC CCAAATCTTC TTCTGCAAGT GGACCATCTG CTAGCATCAA TAGTAGCAGT	420
GTTTCAGCAG GAAGCTACAT GCTGTTCCCA AAGGGATGGC AATGCCTCTG TCAAGGAAAG	480
ACCCAACCTT AAATGCTGCC GATGGGCCTT TGCTTAAAGC CTCAGTGTCC AGCCCTGTGA	540
AAGCATCTTC TTCCCTGTG AGATCCGCTC CATTATCAC TAGAACTGT GAGGTGCAGA	600
GTCCTGAGCT ACTTCACAAA ACTGTTAGTC CTCTGAAAAC AGAGGTGTTG AAACCATGTG	660
AGAAGCCAAC TTTATCCCAG GCACTTCAGC CCAAAGAGGG AGCTAACAAG GAAGTTTGTC	720
TACAGTCACA GTCCAAGGAC AACTTGCAA CACCAGGAGG AAGAGGAATT AAGCCTTTCC	780
TGGAACGCTT TGGAGAGCGT TGCAAGAAC ACAGTAAAGA AAGTCCAAC TGCAGAGCAT	840
TTCATAGAAC CCCAAATATC ACTCCAAATA CAAAAGCTAT CCAGGAAAGA TTATTCAAGC	900
AAAACACGTG TTTATCTAC TACCCCAATT TAGCACAGCA GCTCAAACAG GAGCGTGAAA	960
AGGAACTGGC GTGTCTCCGT GGCCGATTG ACAAGGGCAG TCTCTGGAGT GCAGAGAAGG	1020

ATGAAAAGTC	AAGAAGCAAA	CAGCTAGAAA	CCAACAGGAA	GTTCACTGTC	AGAACTCTCC	1080
CCTCAAGAAA	CACCAAATTG	TCTCAAGGCA	CCCCGTCGAC	CTCTGTGTCA	GATAAAGTGG	1140
CTGAGACTCC	AACCGCAGTG	AAGATTCTCTG	GTACAGAGCC	TGCAGGTTCC	ACTGAAAGCG	1200
AAATGACAAA	GTCCAGCCCT	TTGAAAATAA	CATTGTTTTT	AGAAGAGGAG	AAGTCCTTAA	1260
AAGTAGCATC	AGACCCGGAG	GTTGAGCAGA	AGACTGAAGC	AGTGCATGAA	GTAGAGATGA	1320
GTGTGGACGA	TGAGGATATC	AACAGCTCCA	AGTCATTAAC	GACATCTTCA	GTGANTTCCC	1380
TAGNGGAANG	GGGAACTGGA	CNGTGGAAAA	GANCCAAGGA	GGAGATGGAC	CAAGTGGGGA	1440
ACGGAAAGCA	GCGAGGNGCA	GGAAGATGTG	CNGAATATCT	CCTCAATNTC	TTNACANGNT	1500
CCCCTGGCT	CAGACGGTTC	GGCGTGGTGA	ATCTACAGAA	TGTAATTTCT	TCACCTGAGT	1560
TGGAATTGAG	AGACTATAGC	CTGAGTGCTC	CAAGTCCCAA	ACCAGGAAAA	TTCCAAAGAA	1620
CTCGTGTCCC	CCGAGCAGAA	TCTGGTGACA	GCCTCAGTTC	TGAGGACCGG	GACCTTCTTT	1680
ACAGCATTGA	TGCATATAGG	TCTCAAAGAT	TCAAAGAAAC	AGAACGCCCT	TCCATAAAGC	1740
AAGTGATTGT	TCGAAAGGAA	GATGTTACTT	CAAAATTGAG	TGAAAAGAAT	GGTGTCTTTT	1800
CTGGTCAAGT	TAATATCAAA	CAAAAAATGC	AGGAAGTCAA	TAATGACATA	AATTTGCAGC	1860
AGACAGTGAT	CTATCAGGCC	AGCCAGGCTC	TCAACTGCTG	TGTTGATGAA	GAGCACGGGA	1920
AAGGATCCCT	GGAAGAAGCT	GAGGCAGAAA	GGCTCTTTCT	GANTGCAACT	GAGAAAAGAG	1980
CACTTCTGAT	TGACGAACTG	AATAAGCTGA	AGAGTGAAGG	ACCTCAGAGG	AGAAACAAGA	2040
CCGCTGTGCG	ATCCCAGAGT	GGATTTGCCC	CATGTAAAGG	GTCAGTCACC	TTGTCAGAGA	2100
TCTGCCTGCC	TCTGAAGGCA	GAGTTTGTAT	GCAGCACCGC	GCAAAAGCCA	GAGTCATCGA	2160
ATTACTACTA	CTTAATTATG	CTAAAAGCTG	GGGCTGAGCA	GATGGTGGCC	ACCCCATTAG	2220
CAAGTACTGC	AACTCTCTTA	GTGGTGATGN	CCCTGACATT	CCCCACCACG	TTACCCCNCA	2280
ANGATGTTTC	CAATGACTTT	GAAATAAATG	TTGAAGTTTA	CAGCTTGGTA	CAAAAGAAAG	2340
ATTCCCTCAG	GCCTGAGAAG	AAGAAGAAGG	CGTCCAAGTT	TAAGGCTATT	ACTCCAAAGA	2400
GACTCCTCAC	ATCTATAACT	TCAAAAAGCA	GCCTTCATGC	TTCAGTTATG	GCCAGTCCAG	2460
GAGGTCTCAG	TGCTGTGCGC	ACCAGCAACT	TTACCCTAGT	TGGATCTCAC	ACACTCTCCT	2520
TATCTTCTGT	TGGAGACACT	AAGTTTGCTT	TGGACAAGGT	ACCTTTTTTG	TCTCCGTTGG	2580
AAGGTCACAT	CTGTTTAAAA	ATAAGCTGTC	AAGTGAATTC	AGCTGTTGAG	GAAAAGGGTT	2640
TCCTTACCAT	ATTTGAAGAT	GTTAGTGGCT	TTGGTGCCTG	GCACCGAAGA	TGGTGTGTTC	2700
TCTCTGGCAA	CTGTATCTCT	TACTGGACTT	ACCCAGATGA	TGAGAGGCGA	AAGAATCCCA	2760
TAGGAAGGAT	AAATCTGGCC	AATTGTATCA	GTCATCAGAT	AGAACCAGCC	AACAGAGAAT	2820
TTTGTGCAAG	ACGCAACACT	CTGGAATTGA	TTACTGTCCG	ACCACAAAGA	GAAGACGATC	2880
GAGAACTCT	TGTCAGCCAT	GTAGAGACAC	ACTCTGTGTC	ACCCAAGAAC	TGGCTCTCTG	2940
CAGATACTAA	AGAAGAGCGG	GATCTCTGGA	TGCAGAAACT	CAACCAGGTC	ATTGTTGATA	3000

TTCGCCTCTG	GCAGCCTGAT	GCATGCTACA	AGCCTGTTGG	GAAGCCTTAA	GCCGAGGAGC	3060
TTCTGCACCG	TGAGAGACTT	TGCTAGCTGT	GTCTTCTTAA	GAAGACAGTT	AGAAGCAGCA	3120
GATTTGCAGG	TTGTATTCTA	TGCTTTAAAT	ATAAAAGGGT	ATGTGCAAAT	ATTCACTACA	3180
TATTGTGCAG	TATTTATATC	TTTTCTATGT	AAAAC TTCAC	CCAGTTTGTC	TTGCATT CGT	3240
ACATGTTTGA	CAGTCAAATA	CTAACAATAT	TCATGAGAAT	TGATATCCAT	GCTAAATATA	3300
ACATTAAGAG	TCTTGTTTTA	TAGAAACCTC	ACTAGCCAGT	TATTCATGAC	AAAAACTATT	3360
ATAATCAAGT	TCTGATTGT	CCTTTGGAGC	TGTGGGTTTG	AAGGTATTAA	GGTCTCAAAC	3420
AGAAACATTT	CAGGACATGT	TTAGTAAAGA	GATGAGAAAA	GGCAGCAAAC	ACTAGTTTAA	3480
GCTGCTCAGA	GCTGCTTTCC	GCAGAGCTGT	GGGCAGGACA	CCGTAACATT	TGGGCCTGCA	3540
TAGTCTATGC	TGAAGGGTTA	AGAGTCACAC	AGCTAGTGCT	CACTCTGACC	CTACGTGTGC	3600
AGTGTGGGGC	ACCTTCTCAC	AGTGCTCAGG	CTTTACTTAA	ACAGCTATTT	TTCATGTAGT	3660
TGAGGATCCT	CATTAACATG	TTCAGCCTTT	TCTCTTATAA	CAAGAGCAA	TGTAAATTGG	3720
AAAAACACAT	ACATAAGGAA	TTTCTACCAA	GCTGCTGTGA	CTACTCCTTT	GCTTCCCAGA	3780
GTTCTTGCT	CGTTTTCTTT	TCATGTTGAT	CTAAACACT	TTACAAATCT	GTTTTGAGAT	3840
CACTGAAAAA	TATATAAAGC	TATGCATTCC	CTTTAAAGCC	CAATGCCTTC	TTGCAATTTA	3900
AAAATATTAC	AATGCATGGC	TGCAGTTTTT	AAATAGTCTG	TGTTTCTCCT	CTGACTGTCA	3960
GTTTATTGAT	GGTTTCATTT	ATAAAACACT	AAATTCTATC	ACTTGCCATT	ATATTTCTTA	4020
CTCCATTTAA	ATGTGGGTTT	TCTTATGTAT	ATTATAAAAG	TATTTTATGA	CTCCTACATA	4080
AATAAATAAT	GTGGAATTGT	CNAAANCAAA	AAAAAAAAAA	A		4121

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2059 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACAAACCACC	AAACCACCAA	ACCTGTTTAC	TCAGATTCAT	GGATTGTTCA	CATATGTTTT	60
AACCACTCAC	CCCACCTCAC	AGAGGTGACC	GAACCCAGGA	CTTCAGTCAT	GCTGGGCTAG	120
CCCTGCATCC	ATGAGCTGTG	TGCCCTCAGG	CCCTTGCTTA	AGCTCCTACG	TAGACGTAGA	180
TGTCCTGTTT	TTATTTAAGG	ATTTGAAAAC	CAGTCATGGG	CACCATGATT	TAACACAAAA	240
TACTTCAGTG	TGATGGTCTA	ATTTCTGAA	AATAATTGTT	TGTTCTTCTT	TCAAGGAAAA	300

ACCAAACCTT ATGAATCCGA GCCGAACCTAT TATAAGCCTT AAAATAAGGA GCCGCCCCGCC	360
CCACATCCCA GTCACCCAGT GTTTGAGTTT GGTTGCCCTT TCTCACCTGT GTAATCACAG	420
GGTATACAAT TCATGTTTCT TATGCATGAA ATTAATTTTC TTTCCCTCTG TGGAGTGGGG	480
CTATATTTTA GACAGGTTTT TATTCGTGGA AGCTCTTCAC TGAGAGCAAT ATTTGAAGTG	540
GCTTAAGAAT TTACGTCACA GCATTTATAA ATGATATACC TCAAAGTTAT GCTCCTTTGA	600
TGTCATATAA TGTCTTGAGC AGTTAGGACA GGTTGAGATG TGACATAAGA AAAAGCAGGA	660
TATGTATGTA ATGGATAGGA ATGTCACCTT ACACGTGTGT GTATTTTCTC TGTCCCTAAG	720
ACTTGGTGTA GTGCCAAGCA TACAGTTGGT ATCTAATTTT TGTTGATGGA AAGTGATGG	780
ATTTAGTATA CCTTAAGTGA ATGGTGTAGC TTGTGTAACA ATGTACCCTA TCTCCCCCTC	840
CCTCTCACTT TTTCTTTCAA ATCGCATAAT AAACCCACAG ATTAGATCAG CTTTCTGGGC	900
GGCGACTTCG AAAAGTACTA AATGATCACC GCACAGAAGC CAGCCCTTTG AAACCCCTCAC	960
TGCTTTCACT TGCCTTCTCC CACTTGACTG TCCCTGTGTC CTCTGTCTCT CCAAGGAAGG	1020
TCTAAACTCC TACGTCTTTC GTTAACAAGC AGTTTAATTT TTAAGAAATC TTAACCTTTC	1080
CTGTGCTTGA CACAATTGAC AATCCCTTTC TTCAAGCCCC ACCACTCTGC GTCCTTGAT	1140
CTGGCTTGCT CCTGGGTCTC TTCCTTCTGG TCTCTTCATG TAACCGAAAT ATTAATTCCC	1200
CAGACTTTTC TTTCTTGCTC TAAGTCACTG GACCATACTC TTGTGTAATT TCCATGCAGT	1260
CATCTTATCT TAGCTTCTGT TTTCTGCTG CGGTCACTTG GCTACCTGTT GCCACGTCTT	1320
CAAGGACTCA CTTCTTTTGC GCTCCTCACT TGGTTAGTTT CAGAACATTA CACTGTTCAA	1380
GGTTCTCCAG TTCGCTCTTC TGTCTTCTGC CTGACTATCG GTGTCTACGT TCTGCTGCTT	1440
CTACTCCAAC ATTTCTATCA CTGTCTTTCA ATTTTATTA CAGTTACTCA AAGGATTTC	1500
TGTGTTTATT TTCCCATCTC TGTTGGCCCA GATTACCGAA TTGGGCTTTC TAGAAGCATT	1560
CAGCCTCATC CCTGCTACAG GCAGTTTTAG GAGCTTTTTG GTGAGAGTCT CTGCTTGGA	1620
TCTAAGACCC TCCTCTGTG TTTGCCACTC TGCTCTGATA AGAGTGTTAA AGAGTTTTCC	1680
AGAAGTCCAG AGTTGTAGCC CTCCAGACCT TCGTAGACAC CATATTTGCA TGGAGAGCCC	1740
TAGGCTTCTT CTGGGAAACT CCATGCGTTC TTGAGACTCT GTGACATTAA TTACCCTGGC	1800
CCTTCCTTTG GTCACCATTA TAGTTGCAAC CTACCTCTAT TGAATCACTT ATTGTACTGT	1860
ATATTTTATT TTTTAAAGTG TCCTTTACTA GAATGTGAGC TCCTCAGGGG CAGGCAAAGA	1920
AACTTCATTC ATTTGGCATC TCTATAGCAT AATGTTTGGT ATATGAGCAT TTAATAAATG	1980
TTGAATAAAT TGCTTCACAT GACAGCTGTT CCTCATGGCG GGCGTCTTCA CTGCCTTTGT	2040
TGCAAAACGG GGGGGAAAA	2059

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1987 base pairs

(B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCGAGAGCT CCGCCATGGC CGCTCTCACC CGGGACCCCC AGTTCAGAA GCTGCAGCAA	60
TGGTACCGCG AGCACCCTGC CGAGCTGAAC CTGCGCCGCC TCTTCGATGC CAACAAGGAC	120
CGCTTCAACC ACTTCAGCTT GACCCTCAAC ACCAACCATG GGCATATCCT GGTGGATTAC	180
TCCAAGAACC TGGTGACGGA GGACGTGATG CGGATGCTGG TGGACTTGGC CAAGTCCAGG	240
GGCGTGGAGG CCGCCCGGGA GCGGATGTTT AATGGTGAGA AGATCAACTA CACCGAGGGT	300
CGAGCCGTGC TGCACGTGGC TCTGCGGAAC CGGTCAAACA CACCCATCCT GGTAGACGGC	360
AAGGATGTGA TGCCAGAGGT CAACAAGGTT CTGGACAAGA TGAAGTCTTT CTGCCAGCGT	420
GTCCGGAGCG GTGACTGGAA GGGGTACACA GGCAAGACCA TCACGGACGT CATCAACATT	480
GGCATTGTCG GCTCCGACCT GGGACCCCTC ATGGTGAATG AAGCCCTTAA GCCATACTCT	540
TCAGGAGGTC CCCGCGTCTG GTATGTCTCC AACATTGATG GAACTCACAT TGCCAAAACC	600
CTGGCCCAGC TGAACCCGGA GTCCTCCCTG TTCATCATTG CCTCCAAGAC CTTTACTACC	660
CAGGAGACCA TCACGAATGC AGAGACGGCG AAGGAGTGGT TTCTCCAGGC GGCCAAGGAT	720
CCTTCTGCAG TGGCGAAGCA CTTTGTGCCC CTGTCTACTA ACACAACCAA AGTGAAGGAG	780
TTTGGAATTG ACCCTCAAAA CATGTTCGAG TTCTGGGATT GGGTGGGAGG ACGCTACTCG	840
CTGTGGTCGG CCATCGGACT CTCCATTGCC CTGCACGTGG GTTTTGACAA CTTTCGAGCAG	900
CTGCTCTCGG GGGCTCACTG GATGGACCAG CACTTCCGCA CGACGCCCCCT GGAGAAGAAC	960
GCCCCCGTCT TGCTGGCCCT GCTGGGTATC TGGTACATCA ACTGCTTTGG GTGTGAGACA	1020
CACGCCATGC TGCCCTATGA CCAGTACCTG CACCGCTTTG CTGCGTACTT CCAGCAGGGC	1080
GACATGGAGT CCAATGGGAA ATACATCACC AAATCTGGAA CCCGTGTGGA CCACCAGACA	1140
GGCCCCATTG TGTGGGGGGA GCCAGGGACC AATGGCCAGC ATGCTTTTGA CCAGCTCATC	1200
CACCAAGGCA CCAAGATGAT ACCCTGTGAC TTCCTCATCC CGGTCCAGAC CCAGCACCCC	1260
ATACGGAAGG GTCTGCATCA CAAGATCCTC CTGGCCAAC TCTTGCCCCA GACAGAGGCC	1320
CTGATGAGGG GAAAATCGAC GGAGGAGGCC CGAAAGGAGC TCCAGGCTGC GGGCAAGAGT	1380
CCAGAGGACC TTGAGAGGCT GCTGCCACAT AAGGTCTTTG AAGGAAATCG CCCAACCAAC	1440
TCTATTGTGT TCACCAAGCT CACACCATTG ATGCTTGGAG CTTTGGTCGC CATGTATGAG	1500
CACAAGATCT TCGTTTCAGG CATCATCTGG GACATCAACA GCTTTGACCA GTGGGGAGTG	1560
GAGCTGGGAA AGCAGCTGGC TAAGAAAATA GAGCCTGAGC TTGATGGCAG TGCTCAAGTG	1620

ACCTCTCACG ACGCTTCTAC CAATGGGCTC ATCAACTTCA TCAAGCAGCA GCGCGAGGCC	1680
AGAGTCCAAT AAACCTCGTGC TCATCTGCAG CCTCCTCTGT GACTCCCCCTT TCTCTTCTCG	1740
TCCCTCCTCC CCGGAGCCGG CACTGCATGT TCCTGGACAC CACCCAGAGC ACCCTCTGGT	1800
TGTGGGCTTG GACCACGAGC CCTTAGCAGG GAAGGCTGGT CTCCCCCAGC CTAACCCCCA	1860
GCCCCTCCAT GTCTATGCTC CCTCTGTGTT AGAATTGGCT GAAGTGTGTT TGTGCAGCTG	1920
ACTTTTCTGA CCCATGTTCA CGTTGTTTAC ATCCCATGTA GAAAAACAAA GATGCCACGG	1980
AGGAGGT	1987

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 464 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Gly	Arg	Gly	Trp	Gly	Leu	Leu	Val	Gly	Leu	Leu	Gly	Val	Val	Trp	1	5	10	15
Leu	Leu	Arg	Ser	Gly	Gln	Gly	Glu	Glu	Gln	Gln	Gln	Glu	Thr	Ala	Ala	20	25	30	
Gln	Arg	Cys	Phe	Cys	Gln	Val	Ser	Gly	Tyr	Leu	Asp	Asp	Cys	Thr	Cys	35	40	45	
Asp	Val	Glu	Thr	Ile	Asp	Lys	Phe	Asn	Asn	Tyr	Arg	Leu	Phe	Pro	Arg	50	55	60	
Leu	Gln	Lys	Leu	Leu	Glu	Ser	Asp	Tyr	Phe	Arg	Tyr	Tyr	Lys	Val	Asn	65	70	75	80
Leu	Arg	Lys	Pro	Cys	Pro	Phe	Trp	Asn	Asp	Ile	Asn	Gln	Cys	Gly	Arg	85	90	95	
Arg	Asp	Cys	Ala	Val	Lys	Pro	Cys	His	Ser	Asp	Glu	Val	Pro	Asp	Gly	100	105	110	
Ile	Lys	Ser	Ala	Ser	Tyr	Lys	Tyr	Ser	Lys	Glu	Ala	Asn	Leu	Leu	Glu	115	120	125	
Glu	Cys	Glu	Pro	Ala	Glu	Arg	Leu	Gly	Ala	Val	Asp	Glu	Ser	Leu	Ser	130	135	140	
Glu	Glu	Thr	Gln	Lys	Ala	Val	Leu	Gln	Trp	Thr	Lys	His	Asp	Asp	Ser	145	150	155	160
Ser	Asp	Ser	Phe	Cys	Glu	Val	Asp	Asp	Ile	Gln	Ser	Pro	Asp	Ala	Glu	165	170	175	
Tyr	Val	Asp	Leu	Leu	Asn	Pro	Glu	Arg	Tyr	Thr	Gly	Tyr	Lys	Gly		180	185	190	

Pro Asp Ala Trp Arg Ile Trp Ser Val Ile Tyr Glu Glu Asn Cys Phe
 195 200 205
 Lys Pro Gln Thr Phe Gln Arg Pro Leu Ala Ser Gly Gln Gly Lys His
 210 215 220
 Lys Glu Asn Thr Phe Tyr Ser Trp Leu Glu Gly Leu Cys Val Glu Lys
 225 230 235 240
 Arg Ala Phe Tyr Arg Leu Ile Ser Gly Leu His Ala Ser Ile Asn Val
 245 250 255
 His Leu Ser Ala Arg Tyr Leu Leu Gln Asp Asn Trp Leu Glu Lys Lys
 260 265 270
 Trp Gly His Asn Val Thr Glu Phe Gln Gln Arg Phe Asp Gly Val Leu
 275 280 285
 Thr Glu Gly Glu Gly Pro Arg Arg Leu Lys Asn Leu Tyr Phe Leu Tyr
 290 295 300
 Leu Ile Glu Leu Arg Ala Leu Ser Lys Val Leu Pro Phe Phe Glu Arg
 305 310 315 320
 Pro Asp Phe Gln Leu Phe Thr Gly Asn Lys Val Gln Asp Val Glu Asn
 325 330 335
 Lys Glu Leu Leu Leu Glu Ile Leu His Glu Val Lys Ser Phe Pro Leu
 340 345 350
 His Phe Asp Glu Asn Ser Phe Phe Ala Gly Asp Lys Asn Glu Ala His
 355 360 365
 Lys Leu Lys Glu Asp Phe Arg Leu His Phe Arg Asn Ile Ser Arg Ile
 370 375 380
 Met Asp Cys Val Gly Cys Phe Lys Cys Arg Leu Trp Gly Lys Leu Gln
 385 390 395 400
 Thr Gln Gly Leu Gly Thr Ala Leu Lys Ile Leu Phe Ser Glu Lys Leu
 405 410 415
 Ile Ala Asn Met Pro Glu Ser Gly Pro Ser Tyr Glu Phe Gln Leu Thr
 420 425 430
 Arg Gln Glu Ile Val Ser Leu Phe Asn Ala Phe Gly Arg Ile Ser Thr
 435 440 445
 Ser Val Arg Glu Leu Glu Asn Phe Arg His Leu Leu Gln Asn Val His
 450 455 460

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 558 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Ala Leu Thr Arg Asp Pro Gln Phe Gln Lys Leu Gln Gln Trp
 1 5 10 15
 Tyr Arg Glu His Arg Ser Glu Leu Asn Leu Arg Arg Leu Phe Asp Ala
 20 25 30
 Asn Lys Asp Arg Phe Asn His Phe Ser Leu Thr Leu Asn Thr Asn His
 35 40 45
 Gly His Ile Leu Val Asp Tyr Ser Lys Asn Leu Val Thr Glu Asp Val
 50 55 60
 Met Arg Met Leu Val Asp Leu Ala Lys Ser Arg Gly Val Glu Ala Ala
 65 70 75 80
 Arg Glu Arg Met Phe Asn Gly Glu Lys Ile Asn Tyr Thr Glu Gly Arg
 85 90 95
 Ala Val Leu His Val Ala Leu Arg Asn Arg Ser Asn Thr Pro Ile Leu
 100 105 110
 Val Asp Gly Lys Asp Val Met Pro Glu Val Asn Lys Val Leu Asp Lys
 115 120 125
 Met Lys Ser Phe Cys Gln Arg Val Arg Ser Gly Asp Trp Lys Gly Tyr
 130 135 140
 Thr Gly Lys Thr Ile Thr Asp Val Ile Asn Ile Gly Ile Val Gly Ser
 145 150 155 160
 Asp Leu Gly Pro Leu Met Val Thr Glu Ala Leu Lys Pro Tyr Ser Ser
 165 170 175
 Gly Gly Pro Arg Val Trp Tyr Val Ser Asn Ile Asp Gly Thr His Ile
 180 185 190
 Ala Lys Thr Leu Ala Gln Leu Asn Pro Glu Ser Ser Leu Phe Ile Ile
 195 200 205
 Ala Ser Lys Thr Phe Thr Thr Gln Glu Thr Ile Thr Asn Ala Glu Thr
 210 215 220
 Ala Lys Glu Trp Phe Leu Gln Ala Ala Lys Asp Pro Ser Ala Val Ala
 225 230 235 240
 Lys His Phe Val Ala Leu Ser Thr Asn Thr Thr Lys Val Lys Glu Phe
 245 250 255
 Gly Ile Asp Pro Gln Asn Met Phe Glu Phe Trp Asp Trp Val Gly Gly
 260 265 270
 Arg Tyr Ser Leu Trp Ser Ala Ile Gly Leu Ser Ile Ala Leu His Val
 275 280 285
 Gly Phe Asp Asn Phe Glu Gln Leu Leu Ser Gly Ala His Trp Met Asp
 290 295 300
 Gln His Phe Arg Thr Thr Pro Leu Glu Lys Asn Ala Pro Val Leu Leu
 305 310 315 320
 Ala Leu Leu Gly Ile Trp Tyr Ile Asn Cys Phe Gly Cys Glu Thr His
 325 330 335

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Leu Pro Tyr Asp Gln Tyr Leu His Arg Phe Ala Ala
340                               345           350

Gln Gln Gly Asp Met Glu Ser Asn Gly Lys Tyr Ile Thr Lys Ser Gly
355                               360           365

Thr Arg Val Asp His Gln Thr Gly Pro Ile Val Trp Gly Glu Pro Gly
370                               375           380

Thr Asn Gly Gln His Ala Phe Tyr Gln Leu Ile His Gln Gly Thr Lys
385                               390           395           400

Met Ile Pro Cys Asp Phe Leu Ile Pro Val Gln Thr Gln His Pro Ile
405                               410           415

Arg Lys Gly Leu His His Lys Ile Leu Leu Ala Asn Phe Leu Ala Gln
420                               425           430

Thr Glu Ala Leu Met Arg Gly Lys Ser Thr Glu Glu Ala Arg Lys Glu
435                               440           445

Leu Gln Ala Ala Gly Lys Ser Pro Glu Asp Leu Glu Arg Leu Leu Pro
450                               455           460

His Lys Val Phe Glu Gly Asn Arg Pro Thr Asn Ser Ile Val Phe Thr
465                               470           475           480

Lys Leu Thr Pro Phe Met Leu Gly Ala Leu Val Ala Met Tyr Glu His
485                               490           495

Lys Ile Phe Val Gln Gly Ile Ile Trp Asp Ile Asn Ser Phe Asp Gln
500                               505           510

Trp Gly Val Glu Leu Gly Lys Gln Leu Ala Lys Lys Ile Glu Pro Glu
515                               520           525

Leu Asp Gly Ser Ala Gln Val Thr Ser His Asp Ala Ser Thr Asn Gly
530                               535           540

Leu Ile Asn Phe Ile Lys Gln Gln Arg Glu Ala Arg Val Gln
545                               550           555

```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 229 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```

Met Pro Ser Leu Trp Asp Arg Phe Ser Ser Ser Ser Ser Ser Ser
1           5           10           15

Ser Ser Arg Thr Pro Ala Ala Asp Arg Pro Pro Arg Ser Ala Trp Gly
20           25           30

Ser Ala Ala Arg Glu Glu Gly Leu Asp Arg Cys Ala Ser Leu Glu Ser
35           40           45

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Ser Asp Cys Glu Ser Leu Asp Ser Ser Asn Ser Gly Phe Gly Pro Glu
 50 55 60
 Glu Asp Ser Ser Tyr Leu Asp Gly Val Ser Leu Pro Asp Phe Glu Leu
 65 70 75 80
 Leu Ser Asp Pro Glu Asp Glu His Leu Cys Ala Asn Leu Met Gln Leu
 85 90 95
 Leu Gln Glu Ser Leu Ser Gln Ala Arg Leu Gly Ser Arg Arg Pro Ala
 100 105 110
 Arg Leu Leu Met Pro Ser Gln Leu Leu Ser Gln Val Gly Lys Glu Leu
 115 120 125
 Leu Arg Leu Ala Tyr Ser Glu Pro Cys Gly Leu Arg Gly Ala Leu Leu
 130 135 140
 Asp Val Cys Val Glu Gln Gly Lys Ser Cys His Ser Val Ala Gln Leu
 145 150 155 160
 Ala Leu Asp Pro Ser Leu Val Pro Thr Phe Gln Leu Thr Leu Val Leu
 165 170 175
 Arg Leu Asp Ser Arg Leu Trp Pro Lys Ile Gln Gly Leu Leu Ser Ser
 180 185 190
 Ala Asn Ser Ser Leu Val Pro Gly Tyr Ser Gln Ser Leu Thr Leu Ser
 195 200 205
 Thr Gly Phe Arg Val Ile Lys Lys Lys Leu Tyr Ser Ser Glu Gln Leu
 210 215 220
 Leu Ile Glu Glu Cys
 225

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 232 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Pro Ser Leu Trp Asp Arg Phe Ser Ser Ser Ser Thr Ser Ser Ser
 1 5 10 15
 Pro Ser Ser Leu Pro Arg Thr Pro Thr Pro Asp Arg Pro Pro Arg Ser
 20 25 30
 Ala Trp Gly Ser Ala Thr Arg Glu Glu Gly Phe Asp Arg Ser Thr Ser
 35 40 45
 Leu Glu Ser Ser Asp Cys Glu Ser Leu Asp Ser Ser Asn Ser Gly Phe
 50 55 60
 Gly Pro Glu Glu Asp Thr Ala Tyr Leu Asp Gly Val Ser Leu Pro Asp
 65 70 75 80

Phe Glu Leu Leu Ser Asp Pro Glu Asp Glu His Leu Cys Ala Asn Leu
 85 90 95
 Met Gln Leu Leu Gln Glu Ser Leu Ala Gln Ala Arg Leu Gly Ser Arg
 100 105 110
 Arg Pro Ala Arg Leu Leu Met Pro Ser Gln Leu Val Ser Gln Val Gly
 115 120 125
 Lys Glu Leu Leu Arg Leu Ala Tyr Ser Glu Pro Cys Gly Leu Arg Gly
 130 135 140
 Ala Leu Leu Asp Val Cys Val Glu Gln Gly Lys Ser Cys His Ser Val
 145 150 155 160
 Gly Gln Leu Ala Leu Asp Pro Ser Leu Val Pro Thr Phe Gln Leu Thr
 165 170 175
 Leu Val Leu Arg Leu Asp Ser Arg Leu Trp Pro Lys Ile Gln Gly Leu
 180 185 190
 Phe Ser Ser Ala Asn Ser Pro Phe Leu Pro Gly Phe Ser Gln Ser Leu
 195 200 205
 Thr Leu Ser Thr Gly Phe Arg Val Ile Lys Lys Lys Leu Tyr Ser Ser
 210 215 220
 Glu Gln Leu Leu Ile Glu Glu Cys
 225 230

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 864 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met Ala Met Pro Leu Ser Arg Lys Asp Pro Thr Ser Asn Ala Ala Asp
 1 5 10 15
 Gly Pro Leu Leu Lys Ala Ser Val Ser Ser Pro Val Lys Ala Ser Ser
 20 25 30
 Ser Pro Val Arg Ser Ala Pro Phe Ile Thr Arg Asn Cys Glu Val Gln
 35 40 45
 Ser Pro Glu Leu Leu His Lys Thr Val Ser Pro Leu Lys Thr Glu Val
 50 55 60
 Leu Lys Pro Cys Glu Lys Pro Thr Leu Ser Gln Ala Leu Gln Pro Lys
 65 70 75 80
 Glu Gly Ala Asn Lys Glu Val Cys Leu Gln Ser Gln Ser Lys Asp Lys
 85 90 95
 Leu Ala Thr Pro Gly Gly Arg Gly Ile Lys Pro Phe Leu Glu Arg Phe
 100 105 110

Gly Glu Arg Cys Gln Glu His Ser Lys Glu Ser Pro Thr Cys Arg Ala
 115 120 125
 Phe His Arg Thr Pro Asn Ile Thr Pro Asn Thr Lys Ala Ile Gln Glu
 130 135 140
 Arg Leu Phe Lys Gln Asn Thr Cys Phe Ile Tyr Tyr Pro Asn Leu Ala
 145 150 155 160
 Gln Gln Leu Lys Gln Glu Arg Glu Lys Glu Leu Ala Cys Leu Arg Gly
 165 170 175
 Arg Phe Asp Lys Gly Ser Leu Trp Ser Ala Glu Lys Asp Glu Lys Ser
 180 185 190
 Arg Ser Lys Gln Leu Glu Thr Asn Arg Lys Phe Thr Val Arg Thr Leu
 195 200 205
 Pro Ser Arg Asn Thr Lys Leu Ser Gln Gly Thr Pro Ser Thr Ser Val
 210 215 220
 Ser Asp Lys Val Ala Glu Thr Pro Thr Ala Val Lys Ile Ser Gly Thr
 225 230 235 240
 Glu Pro Ala Gly Ser Thr Glu Ser Glu Met Thr Lys Ser Ser Pro Leu
 245 250 255
 Lys Ile Thr Leu Phe Leu Glu Glu Glu Lys Ser Leu Lys Val Ala Ser
 260 265 270
 Asp Pro Glu Val Glu Gln Lys Thr Glu Ala Val His Glu Val Glu Met
 275 280 285
 Ser Val Asp Asp Glu Asp Ile Asn Ser Ser Lys Ser Leu Thr Thr Ser
 290 295 300
 Ser Val Xaa Ser Leu Xaa Glu Xaa Gly Thr Gly Xaa Trp Lys Arg Xaa
 305 310 315 320
 Lys Glu Glu Met Asp Gln Val Gly Asn Gly Lys Gln Arg Gly Ala Gly
 325 330 335
 Arg Cys Ala Glu Tyr Leu Leu Asn Xaa Xaa Thr Xaa Ser Arg Trp Leu
 340 345 350
 Arg Arg Phe Gly Val Val Asn Leu Gln Asn Val Ile Ser Ser Pro Glu
 355 360 365
 Leu Glu Leu Arg Asp Tyr Ser Leu Ser Ala Pro Ser Pro Lys Pro Gly
 370 375 380
 Lys Phe Gln Arg Thr Arg Val Pro Arg Ala Glu Ser Gly Asp Ser Leu
 385 390 395 400
 Ser Ser Glu Asp Arg Asp Leu Leu Tyr Ser Ile Asp Ala Tyr Arg Ser
 405 410 415
 Gln Arg Phe Lys Glu Thr Glu Arg Pro Ser Ile Lys Gln Val Ile Val
 420 425 430
 Arg Lys Glu Asp Val Thr Ser Lys Leu Ser Glu Lys Asn Gly Val Phe
 435 440 445
 Ser Gly Gln Val Asn Ile Lys Gln Lys Met Gln Glu Leu Asn Asn Asp
 450 455 460

Ile Asn Leu Gln Gln Thr Val Ile Tyr Gln Ala Ser Gln Ala Leu Asn
 465 470 475 480
 Cys Cys Val Asp Glu Glu His Gly Lys Gly Ser Leu Glu Glu Ala Glu
 485 490 495
 Ala Glu Arg Leu Phe Leu Xaa Ala Thr Glu Lys Arg Ala Leu Ile
 500 505 510
 Asp Glu Leu Asn Lys Leu Lys Ser Glu Gly Pro Gln Arg Arg Asn Lys
 515 520 525
 Thr Ala Val Ala Ser Gln Ser Gly Phe Ala Pro Cys Lys Gly Ser Val
 530 535 540
 Thr Leu Ser Glu Ile Cys Leu Pro Leu Lys Ala Glu Phe Val Cys Ser
 545 550 555 560
 Thr Ala Gln Lys Pro Glu Ser Ser Asn Tyr Tyr Tyr Leu Ile Met Leu
 565 570 575
 Lys Ala Gly Ala Glu Gln Met Val Ala Thr Pro Leu Ala Ser Thr Ala
 580 585 590
 Thr Leu Leu Val Val Met Xaa Leu Thr Phe Pro Thr Thr Leu Pro Xaa
 595 600 605
 Xaa Asp Val Ser Asn Asp Phe Glu Ile Asn Val Glu Val Tyr Ser Leu
 610 615 620
 Val Gln Lys Lys Asp Ser Leu Arg Pro Glu Lys Lys Lys Lys Ala Ser
 625 630 635 640
 Lys Phe Lys Ala Ile Thr Pro Lys Arg Leu Leu Thr Ser Ile Thr Ser
 645 650 655
 Lys Ser Ser Leu His Ala Ser Val Met Ala Ser Pro Gly Gly Leu Ser
 660 665 670
 Ala Val Arg Thr Ser Asn Phe Thr Leu Val Gly Ser His Thr Leu Ser
 675 680 685
 Leu Ser Ser Val Gly Asp Thr Lys Phe Ala Leu Asp Lys Val Pro Phe
 690 695 700
 Leu Ser Pro Leu Glu Gly His Ile Cys Leu Lys Ile Ser Cys Gln Val
 705 710 715 720
 Asn Ser Ala Val Glu Glu Lys Gly Phe Leu Thr Ile Phe Glu Asp Val
 725 730 735
 Ser Gly Phe Gly Ala Trp His Arg Arg Trp Cys Val Leu Ser Gly Asn
 740 745 750
 Cys Ile Ser Tyr Trp Thr Tyr Pro Asp Asp Glu Arg Arg Lys Asn Pro
 755 760 765
 Ile Gly Arg Ile Asn Leu Ala Asn Cys Ile Ser His Gln Ile Glu Pro
 770 775 780
 Ala Asn Arg Glu Phe Cys Ala Arg Arg Asn Thr Leu Glu Leu Ile Thr
 785 790 795 800
 Val Arg Pro Gln Arg Glu Asp Asp Arg Glu Thr Leu Val Ser His Val
 805 810 815

Glu Thr His Ser Val Ser Pro Lys Asn Trp Leu Ser Ala Asp Thr Lys
820 825 830

Glu Glu Arg Asp Leu Trp Met Gln Lys Leu Asn Gln Val Ile Val Asp
835 840 845

Ile Arg Leu Trp Gln Pro Asp Ala Cys Tyr Lys Pro Val Gly Lys Pro
850 855 860

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/17296

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 21/04, 14/00; A61K 39/395, 48/00, 38/00; C07K 16/00

US CL : 514/44, 12; 530/387.1, 350; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44, 12; 530/387.1, 350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NOMURA, M. et al. Possible Participation of Autocrine and Paracrine Vascular Endothelial Growth Factors in Hypoxia-induced Proliferation of Endothelial Cells and Pericytes. J. Biol. Chem. 24 November 1995, Vol. 270, No. 47, 28316-28324, especially page 28321.	1-3, 16, 19-20, 24, and 28
A	KATOH, O. et al. Expression of the Vascular Endothelial Growth Factor (VEGF) Receptor Gene, KDR, in Hematopoietic Cells and Inhibitory Effect of VEGF on Apoptotic Cell Death Caused by Ionizing Radiation. Cancer Research. 01 December 1995, Vol. 55, 5687-5692, especially page 5690.	17, 26-31



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 NOVEMBER 1998

Date of mailing of the international search report

23 DEC 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ANNE MARIE S. BECKERLEG

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US98/17296**C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SOKER, S. et al. Inhibition of Vascular Endothelial Growth Factor (VEGF)-induced Endothelial Cell Proliferation by a Peptide Corresponding to the Exon 7-Encoded Domain of VEGF165. J. Biol. Chem. 12 December 1997, Vol. 272, No. 50, 31582-31588, especially page 31582.	14-15, 26-27
Y	O'ROURKE, J. F. et al. Identification of hypoxically inducible mRNAs in HeLa cells using differential-display PCR. Eur. J. Biochem. 1996, Vol. 241, 403-410, especially page 406.	1-2, 7-13, 16-25, 27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/17296

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-31
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 64(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US98/17296

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG-Medline, Scisearch, Biosis, Embase, Derwint, Cancerlit

search terms: hypoxia, differential display, subtractive hybridization, angiogenesis, apoptosis, antisense

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE

2. Where no meaningful search could be carried out, specifically:

They claims could not be searched properly as they include SEQ ID NOS: 1-11 which do not comply with the rules governing nucleic acid and amino acid sequence listing in that no computer readable disk has been submitted with the application. However, the claim language of claims 1-31 was used to conduct a word search, and the relevant references included herein.